The Journal of Comparative Pathology and Therapeutics

Vol. 54. No. 4. OCTOBER, 1944. Price 6 shillings

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ACUTE FATAL DILATATION OF THE STOMACH IN THE DOG (TORSION OF THE STOMACH)

By

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INTRODUCTION

Descriptions of torsion and of acute dilatation of the stomach in the dog with a discussion of causation and treatment of these conditions as separate entities find a place in the better standard works on diseases of the dog. There are however very few cases in the literature in which the morbid anatomy has been adequately described. Cadéac (1908) describes the condition rather fully. Hobday—(McCunn, 1939) mentions only two cases of torsion. Nieberle and Cohrs (1931) give a comparatively full account of the pathology of torsion, as do Muller and Glass (1937) and Hutyra, Marek and Manninger (1938). Dilatation and torsion are described clinically by some of the above authors and there appears to be unanimity that the torsion is a primary mechanical occurrence which is followed inevitably by distension. Acute fatal distension on the other hand is considered to be a functional disturbance.

During August and September, 1942, five dogs were submitted to us for post-mortem examination all of which showed torsion of the oesophagus with rotation and dilatation of the stomach, and with each additional case new and significant facts came to light which cast a doubt on the accepted views on the pathogenesis of gastric torsion. The breed and sex of these animals are given in Table I.

Table I

Case.	*Breed.*	*Age.*	*Sex.*
1.	Great Dane	3 years	Female
2.	Bloodhound	Unknown	„
3.	„	10 years	„
4.	Red Setter	5½ „	„
5.	Great Dane	Unknown	Male

According to the owner of two of the animals, the condition is an accepted cause of loss among bloodhound breeders, she herself having lost five, and her experience suggests that in the case of bloodhounds at least, an hereditary factor may be concerned.

We were impressed by the fact that two distinct anatomical forms of " torsion " are associated with acute gastric dilatation, and that in one of them the course of the splenic artery and vein are peculiarly and possibly significantly altered. These facts have not been mentioned before and it seems to us that based on them

an alternative hypothesis may be put forward in regard to the pathogenesis of gastric torsion. Briefly we believe that the gastric dilatation is primary to and the cause of "torsion."

The paucity of recorded cases, the importance of the condition to bloodhound breeders, the new features observed and the possibility of successful treatment merit description and discussion.

SYMPTOMS AND DIAGNOSIS

The clinical features of the two conditions are similar, the chief being the sudden onset, the excessive size of the abdomen, marked tympany and breathlessness. Large breeds are particularly susceptible.

Symptoms generally commence just after a meal; the animal stands or lies still or moves only with caution. The onset of abdominal swelling is rapid and there is much pain. There may be attempts at vomiting. In a period varying from three-quarters of an hour to three hours, the abdomen becomes grossly distended and there is severe dyspnoea. The animal usually refuses all food or liquid, but if it tries to drink, or if fluid is given forcibly it is regurgitated by way of the nose and mouth.

Percussion gives a marked tympanitic sound, which is of the same metallic note all over the abdomen from the ribs to the pelvis. According to Cadéac (1908) and Hutyra, Marek and Manninger (1938), a dull area about the size of the hand should be found in the right xiphoid region due to the presence of the spleen, but in none of the five cases seen by us was the spleen found in this position. In two it was in the left xiphoid region and in the other three it lay in the left pubic region. According to Cadéac (1908) auscultation reveals no sound, the digestive system being quite inert, and in the thorax, respiratory murmur is heard slightly exaggerated only in the anterior part.

The animal may live for 36 hours after the onset of symptoms, but it may die within a few hours, in a profoundly shocked condition. Jacobs reported a case (cited by Hutyra, Marek and Manninger, 1938), in which the animal lived for five days, and in case three (a bloodhound) with a 90 degrees twist death occurred ten hours after the onset of symptoms. There was a history that this animal had three other attacks of tympany during the previous eight months from which it recovered spontaneously. In the four cases where there was a 270 degrees twist in the oesophagus it does not seem possible that there could have been spontaneous recovery.

In our opinion it is impossible to differentiate clinically between acute primary gastric distension on the one hand and torsion of the oesophagus with distension and rotation of the stomach on the other because the symptomatic pictures would be identical.

TREATMENT

Orthodox treatment consists mainly in preventing asphyxia by trocar puncture, opening the abdomen, and attempting to rectify

the torsion. Puncture alone has led to complete recovery but this would only appear to be possible in simple dilatation, that is one with a 90 degrees twist in the oesophagus. Cadéac (1906) reports one successfully treated case in which, after a slow evacuation of the gas by puncture, the abdomen was incised in the right flank and traction applied upon the intestines and stomach in a caudal direction. Hutyra, Marek and Manninger (1938) suggest that the abdominal incision should be made in the mid-line. Our experiments on normal cadavers have shown that it is easy to correct the 270 degrees torsion when the stomach is deflated if particular attention is paid to the course of the splenic vessels so as to return them to their normal position. The main difficulty when considering treatment would be to determine whether a 270 degrees twist was present. This would only appear to be possible by exploratory laparotomy because of the identity of symptoms of dilatation whether simple or combined with torsion. The condition however, is so serious that such exploratory laparotomy after trocar puncture would be justifiable.

ANATOMY OF THE STOMACH AND RELATED STRUCTURES

The stomach of the dog is freely movable around its point of attachment to the diaphragm, and is fixed cranially by two ligaments. The first, the gastro-phrenic, attaches the region of the oesophageal hiatus of the diaphragm to the cardia. This ligament can have little effect in preventing rotation of the stomach on the axis of the oesophagus, as it is more or less contained within this axis. The second, the gastro-hepatic ligament connects the lesser curvature and the pylorus to the hilus of the liver. This ligament tends to immobilise the pylorus and the lesser curvature of the stomach, but the rotation of the main body of the organ around the axis of the pylorus and oesophagus can still occur. The gastro-duodenal ligament attaches the pyloric part of the stomach to the duodenum and in no way limits the rotation of the stomach. The gastro-splenic ligament is part of the greater omentum and attaches the spleen to the greater curvature of the stomach. This limits the rotation of the stomach only in so far as the length of the splenic vessels limits the area of movement of the spleen. The stomach's only other attachment is to the greater omentum which is freely movable. Movement of the duodenum is limited by the transverse mesocolon, which attaches it to the transverse colon.

The main influences in preventing undue movement of the stomach are the presence of the other organs in the abdominal cavity, mainly the intestines, and the abdominal walls themselves.

AUTOPSY FINDINGS

The autopsy findings fall into two groups ; Group I (cases 1, 2, 4 and 5) showing clockwise torsion of 270 degrees : Group II (case 3 alone) in which simple dilatation was associated with anti-clockwise torsion of 90 degrees.

Group I. The grossly distended and tympanitic abdomen at once attracts attention. On opening the abdomen the stomach is seen filling practically the whole abdominal cavity. The greater omentum is draped tightly over the almost black stomach, which assumes a spherical shape, about 30 centimeters in diameter (in large dogs) on removal of the restraining influence of the abdominal walls.

Examination of the digestive tract shows that the oesophagus has a lengthwise twist near the gastro-oesophageal junction of about 270 degrees in a clockwise direction looking cranially. The greater curvature of the stomach lies just below and parallel to the linea alba, the cardiac portion is cranial and the pyloric part caudal, the latter making an abrupt turn through 180 degrees to pass cranially in the left sublumbar region. Continuing from the pylorus the duodenum passes to the right, ventral to the oesophagus between the liver cranially and the distended stomach ventrally and caudally to regain its normal position on the right side of the body. The splenic vessels pass from their origin to the right and cranially, the first part lying on the right side of the stomach in the region of the gastro-oesophageal junction. They then pass ventrally and to the left crossing the oesophagus in the same position as, but in the opposite direction to the duodenum. Finally they pass along the left side of the stomach to enter the spleen. The disposition of the stomach and the related structures will be seen from Table II and from figures 1, 2 and 3.

The stomach is effectively sealed since the oesophagus has a 270 degrees twist in it, and the duodenum is compressed between the oesophagus dorsally, the liver cranially, and the wall of the distended stomach caudally. No actual tearing of the gastric attachments is evident. The intestines are found pushed into the pelvis and imprisoned dorsally by the stomach, unless they too are distended with gas, when the loops tend to be pushed out around the sides of the stomach. The spleen is much enlarged (43 cm. by 15 cm. weighing 1230 grams, in a Great Dane) and is engorged with blood, its cut surface being almost black. The liver is displaced cranially, its borders nowhere reaching to the caudal border of the last rib. It is pale and bloodless except for the larger vessels which may be congested. The thoracic cavity is reduced in size by the cranial displacement of the liver and diaphragm. The diaphragmatic lobes of the lungs are usually pale with small areas of congestion and emphysema ; the apices of the lungs are congested.

The stomach contains up to a litre of brown fluid, in addition to scanty food remnants, and a large quantity of gas which is more or less odourless. There is marked congestion and oedema of the mucous membrane. The epithelium cannot be seen. Analysis of the stomach contents in case 5 gave the following results : Carbon dioxide 38 per cent., hydrogen 35 per cent., and nitrogen 27 per cent. Total chlorides : 0·454 per cent. Total acid : 0·78 ml.N/10 acid

TABLE II

COMPARISON OF GROUP I AND GROUP II TORSIONS

Structure.	*Position.*	
	Group I	*Group II*
Gastro-oesophageal junction		
Position	Normal	Normal
Direction of twist	The pyloric part of the stomach has passed *ventrally* from right to left	The pyloric part of the stomach has passed *dorsally* from right to left
Degree of twist	About 270 degrees	About 90 degrees
Position of Stomach		
Greater curvature	From the oesophagus it is directed ventrally, then caudally, then dorsally and somewhat to the *left* side of the body	From the oesophagus it is directed ventrally, then caudally, then dorsally and somewhat to the *right* side of the body
Cardiac part	Lies dorsal to the right xiphoid region	Lies dorsal to the right xiphoid region
Fundic part	Lies dorsal to the xiphoid, umbilical and pubic regions	Lies dorsal to the xiphoid, umbilical and pubic regions
Pyloric part	Passes dorsally and then cranially across the *left* sublumbar region to the *left* of the distended stomach	Passes dorsally and then cranially across the *right* sublumbar region to the right of the distended stomach
Lesser curvature	From the oesophagus it is directed caudo - ventrally, and in its pyloric part turns abruptly in a cranial direction to the *left* side of the body	From the oesophagus it is directed caudo - ventrally, and in its pyloric part turns abruptly in a cranial direction to the *right* side of the body
Position of Duodenum		
First part	From the pylorus it continues cranially on the *left side* of the body and crosses the oesophagus ventrally from the left to right xiphoid regions	From the pylorus it continues cranially on the *right side* of the body, and at the level of the gastro-oesophageal junction it turns *sharply* to the right
Remainder	Normal	Normal
Degree of Closure of Orifices of the Stomach		
Oesophageal	270 degrees twist	90 degrees twist
Pyloric	By pressure where the duodenum passes between the oesophagus and the distended stomach	Merely by the kinking of the duodenum where it turns sharply to the right
Position of Spleen	In two cases it lay on the left ventral aspect of the distended stomach in the left xiphoid region. In the other two cases it lay more caudally in the left umbilical and left pubic regions	In the left umbilical and left pubic regions
Course of Splenic Vessels	From their origin they pass ventrally over the oesophagus, close to the point where it is crossed by the duodenum	From their origin they pass dorsal to the distended stomach on the left side

neutralises 100 ml. of gastric contents. Free acid : 0·65 ml.N/10 acid neutralises 100 ml. of gastric contents.

Group II. The abdominal distension and the enlarged stomach are the same as in the other cases ; the surface of the stomach is reddish in colour rather than purple or black. The greater omentum is not draped over the stomach, being torn away at its line of attachment to the greater curvature of the stomach.

Examination of the digestive tract shows that the oesophagus

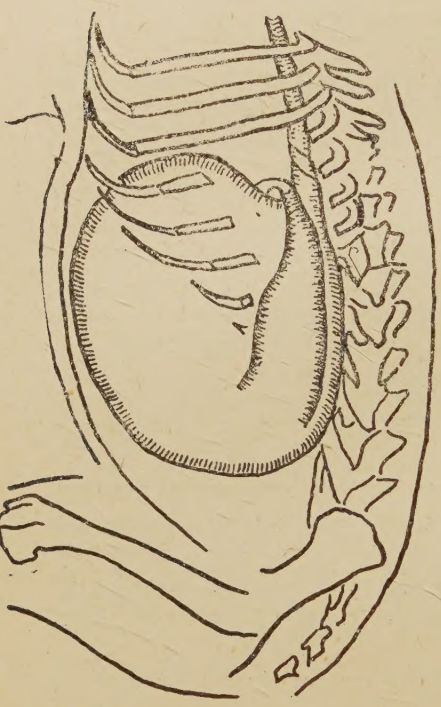

FIG 1. Group I Torsion. Left lateral elevation.

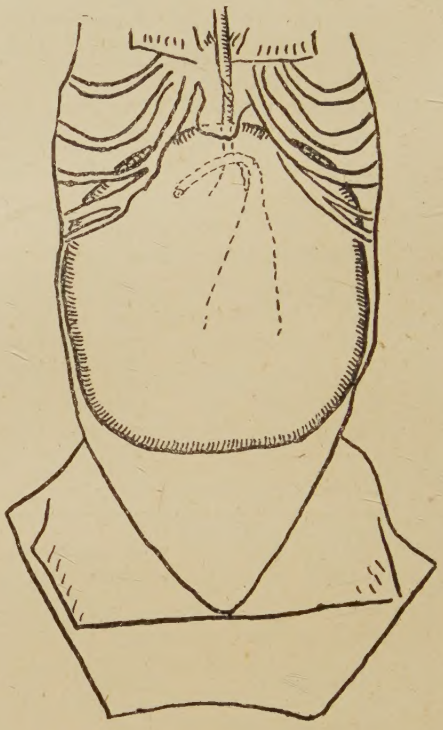

FIG. 2. Group I Torsion. Ventral plan.

has a lengthwise twist near the gastro-oesophageal junction of about 90 degrees in an anticlockwise direction looking cranially. The greatly distended stomach lies in much the same position as in Group I cases but the pylorus turns through 180 degrees and passes in the *right* sublumbar region ; the duodenum lies wholly in the right side. The splenic vessels pass on the left side dorsal to the distended stomach. The disposition of the stomach and the related structures will be seen from Table II and from figures 4, 5 and 6.

As in the Group I cases the stomach is sealed but not to the same extent, there being a 90 degrees twist (in the opposite sense)

on the oesophagus ; at the pyloric end there is only a kink where the duodenum turns sharply to the right. This lesser degree of closure associated with a lesser torsion and the more normal position of the splenic vessels may account for the history of recurrent attacks with spontaneous recovery in this case. The intestines contain a little gas. The rest of the findings are similar to those of Group I

Poenaru & Slava (1906) describe a torsion in which the duodenum passes twice across the oesophagus, which would fit neither Group I nor Group II.

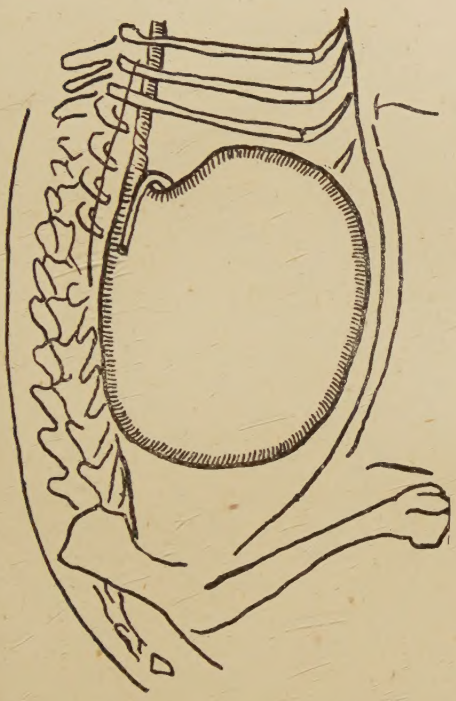

FIG. 3. Group I Torsion. Right lateral elevation.

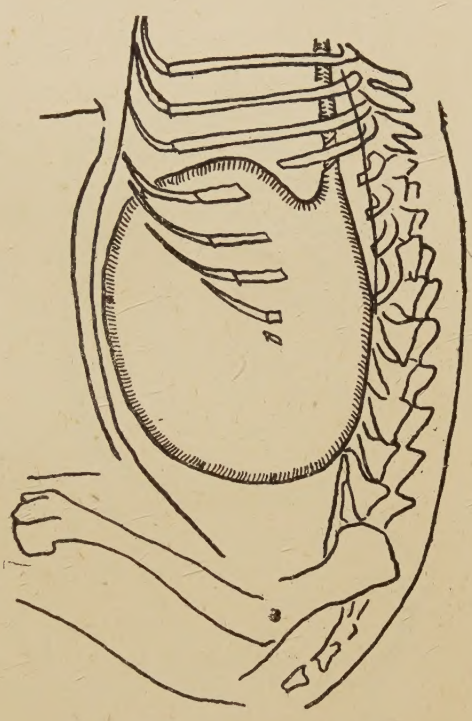

FIG. 4. Group II Torsion. Left lateral elevation.

EXPERIMENTS ON NORMAL CADAVERS

Experiments performed on the dead animal have revealed two possibilities.

(1) If the stomach is lying in its normal position across the abdomen, and is blown up with air by way of the oesophagus, it will twist on the axis of the oesophagus until the greater curvature is ventral, the attachment of the greater omentum being almost parallel to the mid-line of the body, and the pylorus being beside the oesophagus to the right of it. This experiment has been performed on unopened and opened cadavers with the same result. The

resulting position of the stomach is identical to that of Group II dilatation with 90 degrees twist in the oesophagus. The stomach tends to remain distended.

(2) If the organs of the freshly dead cadaver are so orientated that the spleen is lying on the right aspect of the stomach and the splenic vessels so come to lie on its right surface (the spleen may be brought into this position by causing the pyloric part of the stomach to hang ventrally) then dilatation of the stomach with air

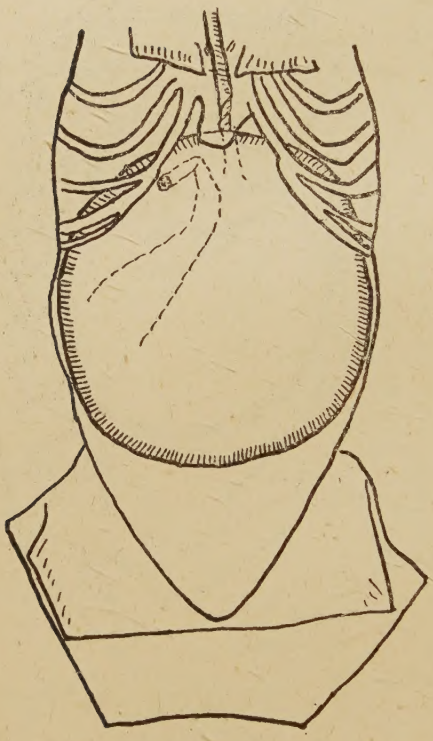

FIG. 5. Group II Torsion. Ventral plan.

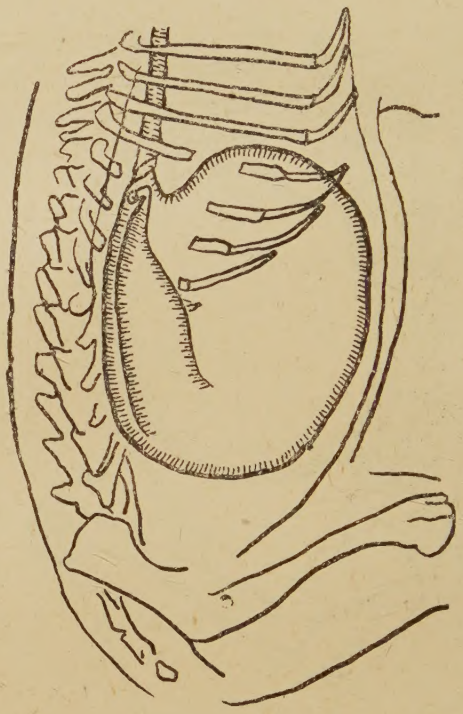

FIG. 6. Group II Torsion. Right lateral elevation.

by way of the oesophagus causes the following to happen : The cardiac part of the stomach balloons out between the pyloric part and the dorsal wall of the abdomen towards the right side, the spleen being carried with it to the right cranially and ventrally. This movement goes on until the spleen is ventral to the cardiac part of the stomach in the right xiphoid region and the splenic vessels have slipped over the right side of the stomach in a cranial direction until they come to lie ventrally across the oesophagus, passing from right to left, and the spleen has again come to lie on the left side of the body.

The spleen is thus dragged in a complete circle by the rotation of the stomach, ending in approximately its normal situation. The pyloric part of the stomach becomes tucked dorsally and to the left of the enlarged cardiac part, the duodenum lying ventrally across the oesophagus from left to right. The greater curvature is now ventral and the line of attachment of the greater omentum lies about parallel to the mid-line of the body. The resulting position of the stomach and splenic vessels is identical to that found in all cases of Group I (270 degrees twist on the oesophagus). If, however, at the moment of commencing the dilatation the spleen does not pass caudally to the stomach but slips laterally to the left of it, a Group II, 90 degrees twist occurs.

The above series of experiments show that in the normal freshly dead cadaver dilatation causes torsion of the stomach in the dog. Whether a Group I (270 degrees twist) or a Group II (90 degrees twist) develops depends on the position of the spleen in relation to the stomach at the moment the dilatation commences.

DISCUSSION

The opportunity of examining five fatal cases of dilatation of the stomach within a period of two months, four with a 270 degrees twist, is remarkable. Three cases were found in the previous ten years protocols of this department. We have since seen one acute dilatation in a lion, and three specimens have been submitted from dogs suspected to have been poisoned which from the symptoms and autopsy findings were undoubtedly acute dilatation. *Post-mortem* examinations and some experiments on normal cadavers have convinced us that the " conditions " of gastric dilatation and gastric torsion are of similar aetiology. Although the conditions present in the cadaver cannot be taken to be the same as those in the live animal it seems probable that whatever is responsible for dilatation will, if the disposition of the spleen and splenic vessels is suitable, give rise to a 270 degrees torsion. Case 3 could be called a 90 degrees anticlockwise torsion but experiments on the cadaver indicate that this is the inevitable result of rapid dilatation of the stomach.

Deliberation on the possible aetiological agents and pathogenesis of torsion of the stomach must take into consideration many points. The commonly expressed explanation of torsion is purely mechanical and may be summarised as follows : The stomach, containing some comparatively small heavy piece of food material, presumably in the pyloric part is pictured as swinging in a pendulum-like fashion : then as a result of a sudden jump from a high bench, or from rolling or playing, the pendulum is swung completely around the point of fixation (the point where the oesophagus passes through the diaphragm) giving rise to a twist. This occludes both the entrance to and exit from the stomach so that gas is produced which cannot escape, giving rise to the distension.

In criticism of the mechanical theory several objections can be raised. Careful questioning of owners showed that sudden or vigorous movement can be eliminated, and in the cases we examined, the stomach contents were not such as to facilitate a pendulum movement. As to the diets of the animals concerned it was only possible to obtain these in two cases (the two bloodhounds). The regular diet of these two animals was $1\frac{1}{2}$ pounds of meat, or rabbit boned, boiled and minced, 6 ounces of stale toasted bread and one biscuit. In both cases the last meal consisted of minced rabbit. In the normally tightly packed abdominal cavity of the dog, the tonicity of the abdominal muscles, the shortness of the gut, and the normal absence of much gas or fluid, all tend to preclude the very free mobility visualised for the stomach. Another observation that may be offered as criticism is the rarity with which one sees a dilated stomach at autopsy. This indicates that the stomach normally contains few, if any, gas producing organisms which by saccharolysis and proteolysis give rise to dilatation. On those rare occasions when gastric dilatation is found the contents are usually bile stained or faecal and there is frequently associated a severe toxaemia. In addition a dog can normally eructate gas with ease.

In view of the above observations we feel that it is more likely that the acute fatal dilatation is a condition precipitated by a meal, often excessively large, which overwhelms a digestive system already weakened, for example, by improper feeding over a prolonged period. (This is common in large breeds, particularly in war-time.) In such animals the gastric contents are in all probability abnormal while sphincter and gastric musculature are almost certainly atonic. Both these abnormalities probably have to coexist before a fatal retention of gas can occur.

SUMMARY.

Five cases of acute fatal dilatation of the stomach of the dog have been autopsied, and found to fall into two groups. Four cases fell into Group I in which there is a 270 degrees clockwise twist in the oesophagus, the pylorus and the first part of the duodenum lying on the left side of the distended stomach, the duodenum and the splenic vessels crossing ventrally over the oesophagus, the former from left to right, the latter from right to left. The other case fell into Group II in which there is a 90 degrees anticlockwise twist in the oesophagus, the pylorus and duodenum being wholly on the right of the distended stomach, and the splenic vessels passing on the left side dorsal to this organ.

From experiments on newly dead cadavers, it is considered that the essential change in both these groups is the same, namely rapid dilatation of the stomach. Whether a Group I or a Group II " torsion " results depends on the position of the spleen at the onset of dilatation.

ACKNOWLEDGMENT

We are grateful to Mr. W. G. Robinson who sent us two of the cases, and also put us into communication with a breeder of bloodhounds, and to Drs. Robertson and Ramsay, who undertook the analysis of the gastric contents.

We are indebted to Mr. J. C. Rennie for his technical assistance and for the illustrations.

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[*Received for publication February 21st, 1944*]

PATHOGENESIS OF *PASTEURELLA SEPTICA* INFECTION IN DEVELOPING CHICK EMBRYO*

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INTRODUCTION

The developing chick embryo with its membranes has been extensively used as a medium for the study of a large number of viruses. Burnet (1936) gave a very good review of the literature on this subject.

Goodpasture (1933) was the first worker to draw attention to the use of both the embryo and the chorion-allantois for the study of bacterial infections. Goodpasture and Anderson (1937)studied the bacterial invasion of the chorion-allantoic membrane of chicks using a variety of organisms, including *Streptococci*, *Br. abortus*, *C. diptheriae* and *M. tuberculosis* (avian). They also studied the protection afforded by diphtheria antitoxin against *C. diptheriae*. The method they used was that described by Burnet (1933) which consisted in creating an artificial air sac, thus exposing a large area of the chorion-allantois to infection.

Gallavan (1937) observed that *H. influenzae* occasionally produced lesions in the brain of the experimental embryos, similar to that of meningitis and ependymitis in children. Gallavan and Goodpasture (1937) made similar observations while working with *H. pertussis*. They observed that lesions similar to those of whooping cough could be seen in the respiratory tract of infected embryos.

All the above authors infected the embryo by dropping suspensions of organisms on to the chorion-allantoic membrane. But Buddingh and Polk (1939a, 1939b, 1939c), in their studies of experimental meningococcus infection, injected their inocula intravenously, intracranially and directly into the amniotic sac and into the body wall, in addition to dropping on to the chorion allantois. They found that the lesions of meningococcus infection in man, namely, a septicaemia, sinuitis, pneumonia and meningitis, could be reproduced in the chick embryo. They also studied the passive immunisation of chick embryos against meningococci by means of serum given intravenously.

Metzer, Beaudette and Stokes (1939) studied the experimental infection of chick embryos with *Br. abortus* and suggested that ten-day-old embryos might be useful for the isolation of *Br. abortus* from the udders of infected cows.

Since then many other organisms have been studied using the chick embryo and its membranes as experimental subjects. Paterson (1940) studied the experimental infection with *Listerella* and observed that the lesions produced closely resembled those in the natural hosts. Chen (1940) made similar studies with *Cl. tetani*, Morrow, Syverton, Stiles and Berry (1938), and also Stefanopoulo and Chevé (1941), cultivated *L. icterohaemorrhagica* on the chorion-allantoic membrane, Swift (1941) similarly grew pleuropneumonia-like organisms, Buddingh and Womack (1941) infected chick embryos with *Bact. tularense*, *Brucella* and *Past. pestis*, while Moore (1941, 1942) has studied lesions produced by *Mycobacteria* and leprosy bacillus on the chorion-allantoic membrane, and Emmart and Smith (1941) made similar studies with the tubercle bacillus.

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* Work done at the Veterinary Laboratory, Ministry of Agriculture, Weybridge, as a part of the Ph.D. thesis of Cambridge University. (1943.)

Chick embryos have also served as culture medium for the cultivation of protozoan parasites. Longley, Clausen and Tatum (1939) successfully cultivated *Trypanosoma rhodesiense*, *T. equiperdum*, *T. brucei* and *T. evansi* in chick embryos, using the diluted infected blood of the embryo to transfer the infection serially from one batch of embryos to the next. About the same time, Levine, Brandley and Graham (1939) reported similar successes with *Trichomonas foetus*, using the allantoic fluid for transfers to fresh embryos. Alture-Webster (1941) reported cultivating *T. equiperdum* in the yolk sac.

The writer has used developing chick embryos in his titration of the pathogenicity of *Past. septica* strains and to study passive immunisation against *Past. septica*. The embryos have proved very susceptible to experimental infection. It was therefore decided to use them again in the study of pathogenesis ; to follow the progressive development of lesions caused by *Past. septica* and also to attempt to find out how *Past. septica* kills its host.

EXPERIMENTAL

Each of 60, 14 day old embryos was inoculated with 0·05 ml. of a 10^{-7} dilution of a suspension of the strain P5 (F) in sterile saline (grown on plain agar slope for 20 hours and washed off with saline) standardised to the opacity of No. 2 tube (Brown's scale) using Burnet's method. At 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 60 and 72 hours after inoculation, three embryos were examined, two bacteriologically and one histologically. The protocol of this experiment (adapted from the chart prepared by Buddingh and Polk, 1939) is set out in Table II.

The inoculation of these 60 embryos took a considerable time and it could be divided into three distinct periods : morning, afternoon and evening. As it happened, almost all of the embryos inoculated in the morning and the afternoon were opened within the period three to 48 hours after inoculation, while the embryos inoculated in the evening were opened at 48 hours to five days. This last lot of embryos happened to survive, because by the time they were inoculated almost all the organisms in the suspension had died. This was shown by the negative results of the viable count. A count was made later under similar conditions and the following results were obtained. (Table I.)

Table I

Viable Count of Suspension in Saline at Intervals

Dilution of Suspension.	*Interval*		
	—	4 *Hours.*	8 *Hours.*
10^{-7}	142, 183, 168 Average = 131	13, 6, 8 Average = 9	0, 0, 0 Average = 0
10^{-6}	Too numerous to count	153, 97, 127 Average = 126	0, 0, 3 Average = 1
10^{-5}	„	Too numerous to count	0, 2, 0 Average = 1

Table II

Examination of Inoculated Embryos

	3 *hours.*	6 *hours.*	9 *hours.*	12 *hours.*	15 *hours.*	18 *hours.*	21 *hours.*	24 *hours.*	30 *hours.*	36 *hours.*	42 *hours.*	48 *hours.*	60 *hours.*	72 *hours.*	4*th* *day.*	5*th* *day.*
Inoculation	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	* * *
0·05 ml. of a 10^{-7}	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	* * ‡
dilution of a sus-	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	‡ 0 0	‡ ‡ ‡
pension of opa-	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	‡ 0 0	‡ ‡	
city Brown's II	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	‡ ‡		
Age of embryo 14 days	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ‡ ‡ *	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ‡ ‡ * 0 0 0	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ‡ ‡ * 0 0 0 ‡ ‡ *	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ‡ ‡ * 0 0 0 ‡ ‡ * 0 0 0	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ‡ ‡ * 0 0 0 ‡ ‡ * 0 0 0 ‡ ‡ *	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ‡ ‡ * 0 0 0 ‡ ‡ * 0 0 0 ‡ ‡ * 0 0 0

However, a complete picture of the development of the lesions from the time of inoculation to the time of death, which was from 36 to 52 hours, was obtained in the case of embryos inoculated in the morning and the afternoon. The results obtained with only these two lots of embryos are considered in the following pages. Embryos which died were opened as soon as possible after death.

DEVELOPMENT OF LESIONS

Chorion-allantoic membrane.—Macroscopic lesions started to appear six hours after inoculation. They consisted of a cloudiness and slight thickening of the membrane and congestion at the site of inoculation. Petechiae were seen at the site of inoculation in one of the three eggs opened at that time. At nine hours, petechiae were seen in two of the three eggs opened. At 12 hours, petechiae were present in all the three eggs opened. Slight exudation was seen at the same time. The formation and spread of petechial haemorrhages and the accumulation of the exudate was progressive until there was a considerable quantity of blood-stained exudate in the case of embryos which died (Fig. 1). Films of exudate stained with Leishman's showed numerous extracellular organisms.

Microscopically, at six hours after inoculation, the cellular reaction was already apparent. The mesoderm was thickened and oedematous and showed a few lymphocytes and polymorphs. This cellular reaction was progressive. At 12 hours, haemorrhages were present on the membrane. Lymphocytes were the predominant type of cell. The mesoderm was infiltrated by lymphocytes and polymorphs, including some neutrophils. Haemorrhages were progressive (Fig. 3). At 24 hours the ectoderm was lost in a diffused layer of cellular elements of blood. There was massive infiltration of the mesoderm by lymphocytes and mononuclear cells. Neutrophils were still remarkably few. The endoderm was also thickened. Beginning at 18 hours, there was a wall of mononuclear cells between the endoderm and mesoderm. The red blood corpuscles appeared by this time to have been almost entirely replaced by lymphocytes and mononuclear cells. The cellular reaction progressed and at 36 hours there was no ectoderm to be seen, but a carpet of lymphocytes, mononuclear cells, a few neutrophils and fewer red blood corpuscles.

There was no evidence of ingestion of bacteria by the blood cells or of bacterial invasion of tissues up to and including 30 hours after inoculation. But in the embryos which died at 30 to 36 hours there were seen colonies of bacteria in the mesoderm, in the wall of the blood vessels and free in the lumen of the blood vessels (Fig. 4).

Liver.—Definite congestion of the liver was seen at nine hours after inoculation. The lesion was progressive and at 12 hours one out of the three embryos showed a fatty liver, while at 15 hours all the embryos had fatty liver. This condition progressed until in the case of the embryos that died, the livers were enlarged and showed a mottled appearance (Fig. 5).

Microscopically, the liver was normal up to six hours. At nine hours cloudy swelling and vacuolation of the liver cells were seen. This condition was progressive and at 12 hours necrosis of the liver cells around the portal and central lobular veins was observed (Fig. 6). Later still, the interlobular spaces became enlarged and the areas of necrosis extended. As late as 30 hours there was still no cellular infiltration. But at 36 hours, that is in the embryos which died, there was a massive lymphocyte infiltration of the parenchyma (Fig. 7).

No bacteria were seen in the liver up to and including 30 hours. At 36 hours bacteria were present, free in the lumen of the blood vessels and in the sinusoids (Fig. 8).

Section of the single heart at 36 hours showed oedema of the muscles and bacteria free in spaces between bundles of muscle fibres (Fig. 9).

Spleen.—The development of lesions in the spleen was parallel to that in the liver. Appreciable enlargement of the spleen was seen at 12 hours after inoculation. The condition was progressive and in dead embryos gross enlargement was present.

No bacteria were seen in the spleen until 36 hours ; the bacteria then found were extracellular (Fig. 10).

Other lesions.—No other regularly occurring lesions were observed. The embryos appeared congested at 18 hours and those that died appeared haemorrhagic. Two of the dead embryos showed haemorrhages on the yolk sac and eight out of the 11 dead embryos had petechial haemorrhages on the pericardium and myocardium (Figs. 5 and 9).

DISCUSSION

Chick embryos have proved to be convenient experimental subjects in which to study the pathogenesis of *Past. septica* infection because the gradual development of the lesions can be conveniently followed.

The reaction of the embryo to the experimental inoculation consisted of a cellular and exudative inflammatory reaction, first seen at the site of inoculation, but later extending further. However, it is notable that neutrophils were very few in number while lymphocytes predominated, a finding which might be due to relatively few neutrophiles being present in the normal chick embryo.

The most notable feature was the lack of phagocytosis. Yet bacteriological examination showed that no bacteria could be demonstrated in the allantoic fluid or the heart blood up to and including 30 hours after inoculation. Neither could any bacteria be seen in sections of liver and spleen up to that time, although pathological changes could be seen in these organs as early as nine hours after inoculation. This suggests a progressive toxaemia, the bacterial invasion of the blood stream occurring immediately before or after death. The fact that two living embryos opened at 48 hours

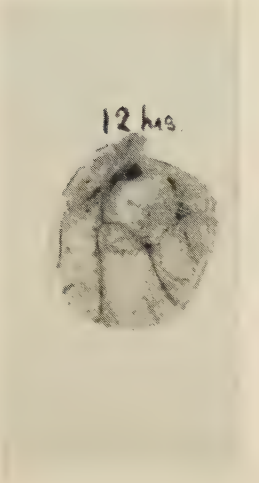

FIG. 1. Petechial

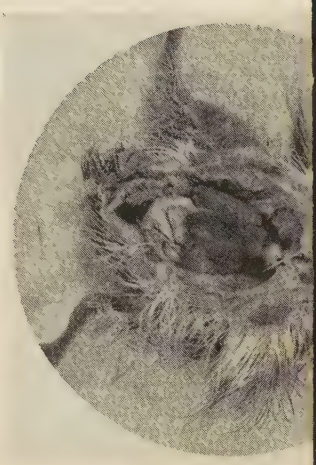

FIG. 5. Chick embryo, whic
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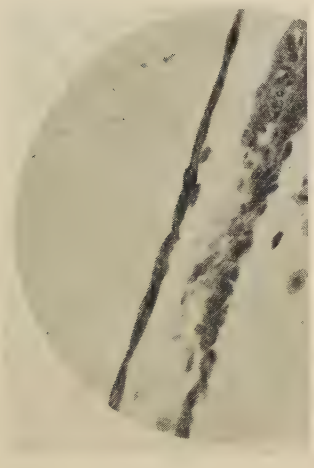

FIG. 2. Section of normal cl
membrane. Stained H &

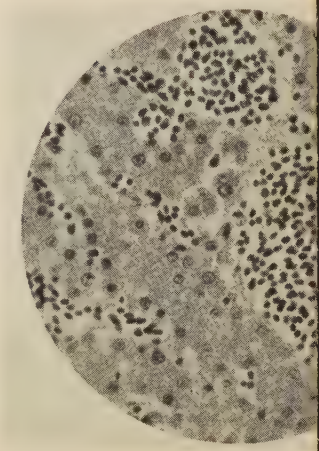

FIG. 7. Section of liver of an
at 36 hours showing lymphocy
Stained H & E × 1,

FIG. 4
after
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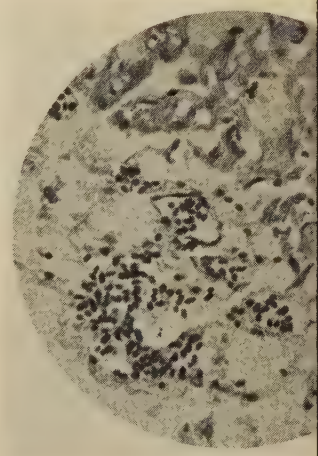
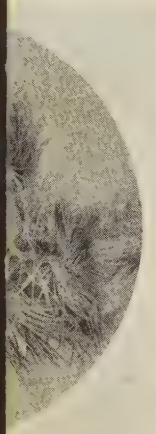


FIG. 9. Section of heart of ar
at 36 hours showing oedema
rhages. Stained H & E



n died at 36
wing mottled
the heart.

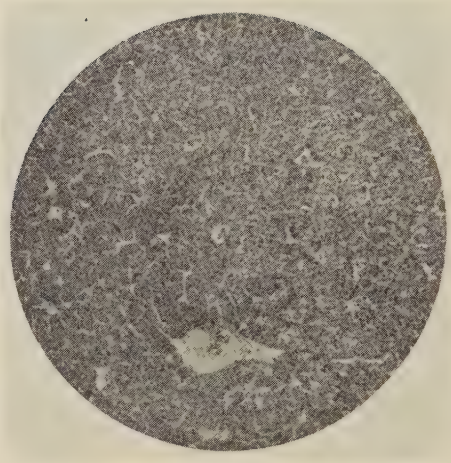
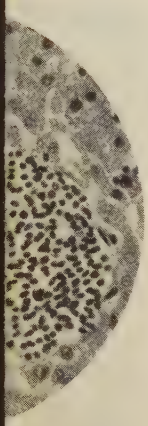


FIG. 6. Section of liver at 12 hours showing necrosis. Stained H & E × 400.



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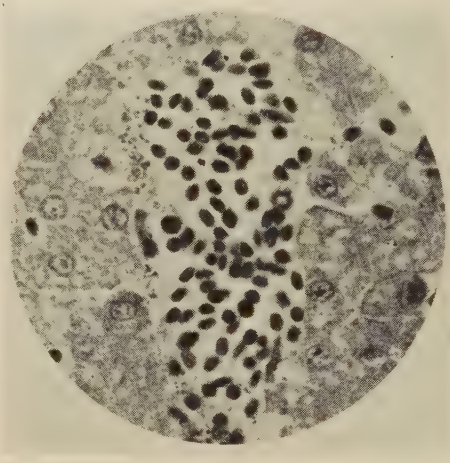
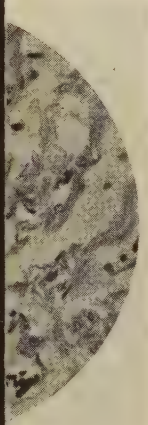


FIG. 8. Section of liver of an embryo dead at 36 hours, showing bacteria in the blood vessels and sinusoids. Stained Gram × 1,000.



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and haemor-
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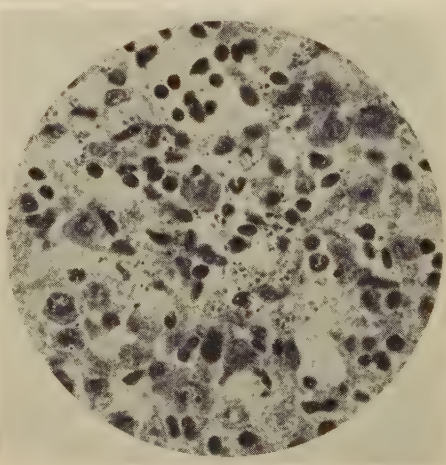

FIG. 10. Section of spleen of an embryo dead at 36 hours, showing bacteria in spleen. Stained Gram × 1,000.

gave positive cultures from both the heart blood and the allantoic fluid suggest that the bacterial invasion of the blood stream took place in these embryos just before death and the fact that one embryo which died and was opened at 60 hours gave a positive culture from the heart blood but a negative culture from the allantoic fluid suggests that the latter was infected via the blood stream and not through the membrane.

But so far, no worker has succeeded in demonstrating *in vitro* the production of toxins by *Past. septica*. However, this does not preclude the possibility of toxin production *in vivo*. Whilst the results obtained in this experiment suggest some kind of toxic action, much further work is needed before any conclusion can be reached.

Although no positive results were obtained in this experiment, the observation that the bacteria were not phagocytosed by the leucocytes confirms the observations made by De Moulin (1938) who worked with a strain of *Past. boviseptica*.

SUMMARY

1. A review of the recent literature on the use of the chick embryo and its membranes in the study of bacteria is given.

2. A description of an experiment made to study the pathogenesis of a strain of *Past. aviseptica* in chick embryos and the results obtained is given.

3. The results are briefly discussed. It is suggested that a toxin is possibly produced *in vivo* by the organism.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the author's indebtedness to Professor T. Dalling, M.A., M.R.C.V.S., for the permission given to the author to work at the Veterinary Laboratory, Weybridge. To Mr. A. Norman, chief technician of the same laboratory, the author is indebted for the photographs, and to Mrs. J. Pentelow of Wakefield, for typing the scri t.

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[*Received for publication June 8th, 1944*]

STUDIES ON THE HAEMATOLOGY OF SHEEP

3. LEUCOCYTIC REACTIONS

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INTRODUCTION

The leucocytic picture has become established as an aid to diagnosis and prognosis, but the degree of its value—as Harvey (Harvey and Hamilton 1932) pointed out—is still a matter of controversy. This article is confined to a description of various types of reaction and to setting arbitrary standards where these appear to be practical.

The technique used in this investigation and the results obtained from healthy sheep were given in the first article (Holman, 1944a), but for convenience the results for leucocytes are repeated as percentiles, in which the figure limiting each 10 per cent. is given; the limit excluding the first 5 per cent. at each end has also been indicated, the respective figures being given in brackets (Table 1).

In the second article (Holman. 1944b), it was pointed out that the wide distribution of the normal range allowed small reactions to be overlooked and a Maximum Admissible Difference was calculated for each type of cell. M.A.D.s for daily variation were included and it was accepted that a variation greater than this coefficient was to be taken as evidence that the normal biological variation over 24 hours had been disturbed. These coefficients and the ones for error have been appended to Table 1.

Table 1.

Distribution and Variation of Leucocytes.

	Percentiles													*Maximum Admissible Difference*	
														Error	*D.V.*
Band ..	0	(0)	0	0	0	0	1	1	1	2	3	(3)	5	—	1·0
Poly. ..	11	(13)	14	16	19	20	21	23	26	29	39	(41)	47	11·9	15·6
Lym. ..	41	(46)	53	58	63	66	68	70	72	74	77	(79)	83	13·6	16·0
Mono. ..	0	(0·5)	1	2	2	2	3	4	5	6	7	(9)	15	5·0	3·3
Eos. ..	0	(0)	0	0·5	1	2	3	4	5	6	9	(11)	15	4·2	7·8
Bas. ..	0	(0)	0	0	0	0	0·5	1	1	1	1	(2)	3	1·5	1·6
W.c. 10^3..	1·0	(5·1)	6·0	6·8	7·7	8·5	9·0	9·5	10·7	11·5	12·2	(12·8)	17·8	2·3	4·0

Additional figures, limiting the first 5 per cent. at each end, are given in brackets. D.V. refers to daily variations.

TYPICAL REACTIONS

Schilling (1929) formulates the fundamental leucocytic reaction to infections or other irritations, as consisting of three phases. The first phase is the neutrophilic invasion phase (or battle phase) in which there is an increase in the number of neutrophil cells, their numbers including an increased proportion of metamyelocytes (band forms and possibly young forms) a change known as a " shift to the left," while the eosinophils tend to disappear. The second

phase is termed the phase of monocytic defence; here the neutrophil cells recede in numbers and show a smaller proportion of immature forms, a small number of eosinophils return and the monocytes increase in numbers to above the normal level. The third phase is the phase of lymphocytic recovery; here with the decrease in neutrophils there is a relative lymphocytosis accompanied by an eosinophilia.

Working on sheep it has been found that these phases are similar to, but not identical with, those described by Schilling. The invasion phase is precisely similar but the defence phase is better classified by the decrease in the neutrophil reaction rather than by an increase in monocytes, for this is irregular in its appearance, and similar increases may occur in the invasion phase or in the recovery phase. In contrast to man, the recovery phase is different for sheep, the stage of relative lymphocytosis is not apparent and a small increased proportion of neutrophil cells may persist for days or even weeks after the recovery of the animal. This phase is therefore better classified by the appearance of eosinophils in numbers above the average or by an eosinophilia, often accompanied by the appearance of one or two basophils. Examples of different degrees of reaction caused by experimental injections of *Staphylococcus aureus* are depicted in Fig. 1, and these will now be discussed.

Mild Transient Reaction

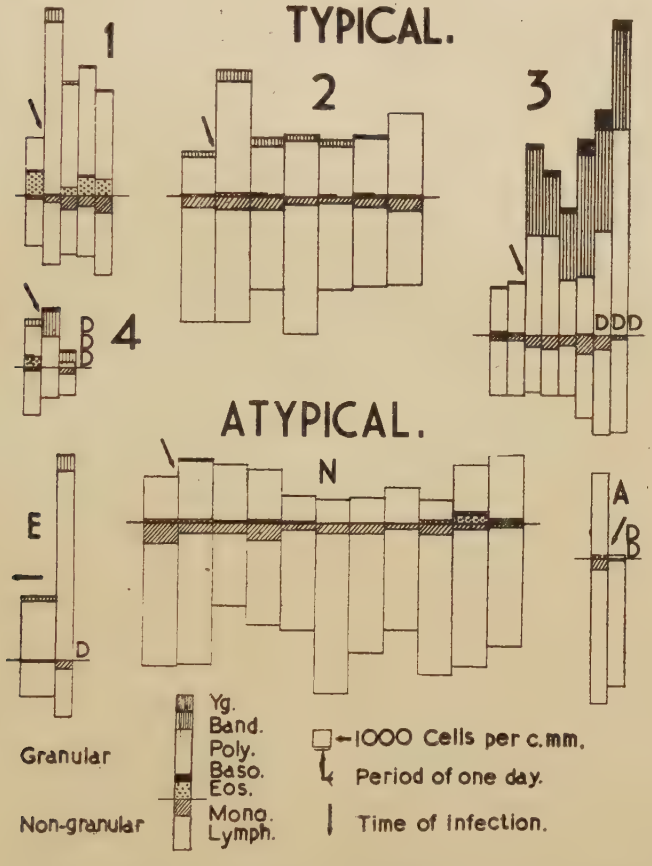
	Differential Counts				
Types of Experiment ...	1st	2nd	3rd	4th	5th
Band forms	0	6	2	1	1
Polymorphs	31	74	59	56	47
Lymphocytes	43	26	26	27	33
Monocytes	3	3	8	5	9
Eosinophils	22	1	5	10	9
Basophils	1	0	0	1	0
W.C. 10^3	6·3	14·0	10·6	11·2	10·8

Pre-inoculation. The sheep appeared healthy and the leucocytic picture (see above) was normal except for the very high eosinophil count which was assumed to be due to worm infestation. The animal was injected subcutaneously with a small amount of pus containing staphylococci taken from an abscess in a sheep.

Invasion Phase. On the second day the sheep was dull and showed a temperature of 104·8° but retained its appetite. The total leucocyte count remained just within the range shown in Table I, but had increased by over two M.A.D.s for daily variation, a circumstance considered to be highly significant. Both polymorphs and band forms had increased to figures outside the limits in Table I, indicating a relative neutrophilia with a shift to the left. The monocytes showed no change, but the eosinophils had decreased to only 1 per cent., a change exceeding twice the M.A.D. and therefore highly significant. (Fig. 1, example 1.)

Defence Phase. On the third day the sheep appeared healthy but its temperature was 104°. The total count had fallen to well within the normal range and the neutrophilia was receding with a decrease in metamyelocytes; the monocytes showed an increase —considered just significant—and there was a slight increase in eosinophils.

FIG. 1



It is regretted that owing to technical difficulties in reproduction low eosinophile counts have appeared as a black line.

Recovery Phase. On the fourth day the sheep appeared normal with a temperature of 103·6°. The total count was roughly the same but there was an insignificant decrease in the neutrophilia and in the proportion of monocytes. The recovery phase was denoted at this time by the increase in eosinophils which returned to a high figure. On the fifth day there was little change but the neutrophils had now decreased to a point just within the highest limit of the normal range and the eosinophils remained at a high level.

Mild Prolonged Reaction

The figures for this reaction were published in detail as differential counts (Taylor, Holman and Gordon, 1941). They are given here as a composite diagram consisting of the averaged absolute counts for three sheep. (Fig. 1, example 2.) The sheep were injected subcutaneously with 1 ml. of a culture of *Staph. aureus* matched to No. 2 tube of Brown's scale.

The pre-experimental blood picture was normal, having a total count of 11·0 thousand with 33 per cent. neutrophils of which 1·6 per cent. were band forms. No blood sample was taken on the day following inoculation and therefore the peak of the invasion phase may have been lost, but on the second day after inoculation the neutrophilic reaction was marked with a total count of 14·7 thousand and a neutrophil percentage of 48, of which seven were band forms. The mildness of the reaction, suggested by the smallness of the "shift to the left," is confirmed by the continued presence of the eosinophils, and it can be seen from Table I that the only significant changes are the increase in the total count by an amount exceeding the limit set by the M.A.D. for daily variation and an increase in the number of band forms to a point just outside the normal range.

The sample two days after this showed the defence phase, for neutrophilic reaction had died down and only 4·7 per cent. of band forms were present. Thus on this day, taken by itself, there was no abnormality, for eosinophils were still present and no monocytic defence period was apparent. The only definite change in the next two samples was the tardy occurrence of a complete eosinopenia at a time when eosinophils would be expected to reappear. In the two samples covering the next four days there was an undramatic return of eosinophils and, as further samples showed no marked increase in these cells, this was accepted as the commencement of the recovery phase.

Although the samples from three sheep have been treated together, in order to show that the different phases are a general rule rather than the spectacular exception, this treatment suffers from two defects. Firstly, samples were taken on alternate days only, and secondly, small changes in individual sheep are obliterated. Thus at the third sample two sheep showed 10 per cent. monocytes which could be accepted as a monocytic defence period and it could be argued that, had the sheep been bled daily, the third animal would have shown this reaction either the day before or after the others. Records from other sheep, however, suggest that the appearance of this monocytic increase is irregular and unreliable. The picture for this reaction makes it clear that although a reaction may be obvious when serial samples are examined, the abnormality would not be evident if samples had been taken at certain periods within the reaction.

Severe Neutrophilic Reaction with Unfavourable Course

The data for both this and the following example have been

taken from unpublished work on lambs that was carried out in co-operation with Dr. A. McDiarmid. All lambs were injected intravenously with thick suspensions of *Staph. aureus*. In each case the figures for three lambs have been averaged.

Two preliminary bleedings have been depicted (see Fig. 1, example 3), as, by chance, no eosinophils were apparent in the pre-inoculation samples. On the day following injection a severe neutrophilic reaction was apparent with a large proportion of metamyelocytes which included a small percentage of young forms. There was also a complete eosinopenia. For the next two days the neutrophil count decreased both relatively and absolutely, but the blood picture remained at the invasion phase in that the proportion of immature forms was increasing, and had even reached a point at which they represented more than half the total neutrophilic cells. It is interesting to note that at this point—when the large proportion of metamyelocytes predicted an unfavourable termination to the infection—all three sheep were still able to stand and retained some appetite. From this day onward there was an increase in the total count but the unfavourable course was quite obvious from the high proportion of immature neutrophils and the steady increase of the young forms. On the next day one lamb was dead but the intense reaction was still obvious in the remaining lambs; the second sheep died the next day and the third lamb died the day after that, with a final sample showing an agonal leucocytosis in which the neutrophilic reaction was still pronounced. Thus, from first to last the reaction remained within the neutrophilic battle stage as shown by the intense " shift to the left " and the complete absence of eosinophils.

Severe Reaction Leading to Fatal Leucopenia

This sample is typical of an overwhelming infection in which the neutrophilia, expected to appear in the peripheral blood, is obliterated, and the only evidence of neutrophilic reaction is that given by the marked " shift to the left." (Fig. 1, example 4.) The pre-inoculation sample shows a fairly high percentage of band forms—not abnormal for young lambs—but after injection there was a pronounced shift and this, together with the complete absence of eosinophils, suggested an unfavourable prognosis. The following day, despite the shift to the left, both neutrophils and lymphocytes had decreased in absolute numbers, and this was accepted as the beginning of a fatal leucopenia. The three lambs were found dead on the following morning.

ATYPICAL REACTIONS

Neutropenia

This reaction results from an infection with tick-borne fever, described elsewhere (Taylor *et al.*, 1941), and is a composite picture of the figures obtained from three sheep. It can be seen that in

the sample taken two days after infection there was a slight increase in neutrophil cells, but that this increase did not amount to a neutrophilia, nor could the appearance of 1 per cent. band forms that accompanied it be termed a shift to the left. (Fig. 1, example N.) Over the next six days there was a steady fall in neutrophil cells but eosinophil cells were still present and the monocytes did not exceed the figures for the pre-inoculation sample. At the next sample the reaction was more marked, with only 13 per cent. polymorphs and a complete absence of eosinophil cells: this phase lasted over the next four days, after which eosinophils were again apparent and polymorphs increased in numbers.

The above example, like example 2, loses some individual reactions owing to the averaging of the figures for three sheep, and by its restriction to samples made on alternate days. Thus it could be suggested that a true neutrophilia was present on the day following inoculation, and this in fact is sometimes the case, but it is an exception to the rule. Again it could be claimed that there was no neutropenia for the neutrophils did not fall below 13 per cent., a point within the normal range, but individual records show that two sheep fell to 5 per cent. and 6 per cent. neutrophils respectively, although the figures for the third sheep merely receded to below the normal average. If, however, the example represented the reaction in a single sheep—as it might well do—the only points of significance would be the fall in neutrophils between the fourth and fifth sample which is in excess of the M.A.D. for daily variation in neutrophils, and the complete absence of eosinophils for three consecutive samples which is unlikely to have occurred by chance.

Agranulocytosis

The production of an agranulocytosis by the toxin of *Cl. welchii*, type A, has been described elsewhere (Gordon, Stewart, Holman and Taylor, 1940). Such an agranulocytosis is depicted in Fig. 1, example A, which consists of the averaged figures for two sheep. It shows that after the injection of toxin the granular cells are almost obliterated and there is a decrease in the number of monocytes, although the lymphocytes remain comparatively unchanged. Comparing this picture and the neutropenia of tick-borne fever with the leucopenia produced by massive doses of staphyloccoci in example 4, it can be seen that in the atypical example there is neither a marked " shift to the left " nor a decrease in lymphocytes.

Reaction Limited to a Terminal Eosinopenia

Some infections, including amongst others, scrapie, produce no change in the blood-picture except during a brief period before death. The terminal counts of a sheep that had been affected with scrapie for some months are depicted in Fig. 1, example E. The sample, taken three days before death, showed no abnormality other

than the absence of eosinophils (a factor that is not significant if limited to a single sample), and the last sample showed, in addition to the eosinopenia, the beginning of an agonal leucocytosis.

ABNORMAL VARIATIONS IN LEUCOCYTES

Neutrophilic Reactions

Neutrophilic reactions are by far the most important from the diagnostic point of view and they will be dealt with in detail.

A mild, transient reaction has been described (Example 1) and proved significant, but for the purpose of setting standards it is desirable to know the absolute minimum that can be regarded as a neutrophilic reaction. The difficulty of setting such a limit for daily samples is seen in Table II, where the results of unpublished work on skin reactions in Johne's disease carried out in collaboration with Dr. A. Wilson Taylor, are summarised. It can be seen

TABLE II

RESULTS 24 HOURS AFTER INTRADERMAL INJECTION OF JOHNIN

	Neutrophilic Bands.		*Reaction Polys.*		*Eosinophils*	
	Sig.	*V.*	*Sig.*	*V.*	*D.*	*I.*
Positive skin reactions	9†	7	11*	5	16	0
Negative „ „	1	4	0	5	3	2
Uninjected controls ...	0	4	0	4	3	1

Sig.—Indicates an increase exceeding the M.A.D. (95 per cent.) for daily variation. V.—Indicates a variation either upward or downward, but within the limit of the M.A.D. D.—Indicates a decrease in the numbers of eosinophils. I.—An increase in the number of eosinophils.

* Four of these were outside the normal limit and therefore could also have been classified as a relative neutrophilia.

† None of these were outside the normal range, the highest being 3 per cent. band forms.

from the table that the standard denoting a reaction must be an arbitrary one and open to error, but that unless it is placed to cover all increases exceeding the M.A.D., some slight reactions will be lost. Thus, with daily sampling it is advisable to take any increase above 15·6 polymorphs in the differential count as a neutrophilic reaction.

Both Schilling (1929) and Wirth (1931), depicting the fundamental curve of neutrophilic reaction in man and the horse respectively, show a small primary decrease in the number of neutrophils just after the time of infection and preceding the much more obvious neutrophilic increase. Further, Wallbach (1935) records a transient decrease in neutrophil cells in rabbits following the injection of toxic material to a susceptible animal, but claims an immediate increase in these cells where the rabbit has been immunised against the antigen.

Using *Cl. welchii* type D toxin, an attempt was made to confirm these points. After injection of the toxin sheep were bled at frequent intervals, 5, 15 and 60 minutes after commencing. The results are summarised in Table III. It can be seen that a small fall in neutrophil cells is common during the first 5 to 15 minutes but that the only occasions when the change was large enough to be considered significant was where the toxin was given subcutaneously. No correlation was noted between the amount of toxin and the extent of the decrease, nor did the decrease distinguish between susceptible animals and those previously immunised.

This experiment showed further that a neutrophilic reaction was often present at the end of the first hour, either as a relative neutrophilia or as a " shift to the left," and that at three hours a combination of these two reactions could be present. Thus reactions can occur very quickly, and for such reactions as anaphylactic shock

Table III

Decrease in Neutrophil Cells following Injections

	Decrease		*No. Change*	*Increase*	
	Sig.	*V.*		*Sig.*	*V.*
Subcutaneous injection of solution of *Cl. welchii* type D toxin	5	4	1	4	0
Intravenous injection of solution of *Cl. welchii* type D toxin	0	7	0	2	0
Subcutaneous injection of 100 ml. innocuous fluid	0	2	0	0	0
Totals	5	13	1	6	0

Sig.—Indicates an increase or decrease greater than the M.A.D. (95 per cent.) for error alone, that is, an alteration of 12 or more cells. V.—Indicates that the alteration was within one M.A.D. All changes measured 5 or 15 minutes after injection.

when the assaulting dose is given intravenously, it would be necessary to take several samples on the first day, for if left till the next day the leucopenia would be overlooked and only the succeeding leucocytosis would be recorded.

The exact borderline between health and disease cannot be drawn but it is advisable for practical purposes, and even at the expense of an occasional error, to try to set standards for the limits of normality, particularly as failure to strike the exact limit would merely establish an unusually high normal value for neutrophils (for example), as a " slight neutrophilia," a wrong diagnosis that would lead to no grave error in prognosis. Therefore, when the technique described in the first article is carried out, the following standards are offered as a guide.

Neutrophilic Reaction.—This can be assumed if there is a rise of at least 16 polymorphs or at least two band forms between the differential counts of two daily samples. It should be noted that

although the M.A.D. for daily variation in band forms is one, the M.A.D. for error alone in basophils is 1·5. The distribution of these two cell types is very similar, and hence it is safer to raise the limit to two cells, as above.

Relative Neutrophilia and Relative Neutropenia.—The limits set for polymorphs in Table I, namely, above 47 and below 11 per cent., are suitable, in that at both extremes the polymorphs represented the total neutrophil count.

Absolute Neutrophilia and Absolute Neutropenia.—As in the above the absolute limits for polymorphs are suitable for this range; for 113 sheep these were 5·6 and 0·7 thousand per c.mm.

For general purposes the relative counts are more sensitive. Both for lambs and sheep, 6 per cent. band forms may be taken as a slight "shift to the left." In moderate reactions about one-third of the neutrophil cells may be band forms, and perhaps 0·5 per cent. young forms may be present. In severe reactions the immature forms constitute more than half the total neutrophil cells and here, as in man, the prognosis is unfavourable. An exception to this rule exists in the case of sheep suffering from a concomitant infection of tick-borne fever, which can be diagnosed by seeing the specific bodies lying within the neutrophil cells during the febrile part of the disease; in this case the band forms easily outnumber the few polymorphs that are present without connoting an unfavourable prognosis.

Toxic changes are sometimes seen in neutrophil cells but they are not as common or as obvious as in the horse. They have not been studied in this investigation.

Lymphocytes

The numbers of lymphocytes are an essential requirement for the estimation of the proportion of other cell types, but they appear of little importance in themselves. In parts of this investigation the proportion of large to small lymphocytes, the lymphocytic-monocytic ratio, the appearance of cells with two nuclei and the proportion of cells showing azure granules were recorded, but the significance of the results could not be interpreted.

The rarity of lymphatic leukaemia in sheep makes the definition of an absolute lymphocytosis of little value and the fall of both neutrophils and lymphocytes in severe infections or anaphylactoid shock can be estimated as easily from the total leucocyte count. In view of this the definition of standards limiting normality appears redundant.

Monocytes

In sheep, these cells are sometimes difficult to distinguish from large lymphocytes but, in contrast to the horse, they are easily distinguished from band forms that have undergone toxic changes. They may show the presence of tick-borne fever bodies during an

infection, and occasionally what appears to be a typical monocyte may contain the remains of an erythrocyte within its cytoplasm.

Monocytes fluctuate during infections, showing increased numbers over short periods (monocytic defence periods), but the significance of these waves is not understood, nor would the error in estimating the number of monocytes permit them to be used as a sensitive diagnostic agent.

Eosinophils

Schilling places great reliance on the absence of eosinophils as a sensitive indication of ill health, and takes the precaution of also examining a thick blood film for the presence of these cells as an additional guide to their numbers. In sheep the significance of the eosinophil count is always complicated by the possibility of worm infestation, and before discussing the standards to be adopted it is advisable to discuss the reaction that usually occurs under the influence of eosinotactic substances. Weinberg and Seguin (1914, 1915) and Biggart (1932) have shown that the production of a local eosinophilia is carried out at the expense of blood eosinophils causing a temporary eosinopenia. The stimulus also leads to an increased production of eosinophil cells, creating a period of eosinophilia. Finally, when the stimulus is neutralised, the eosinophilia dies away, in many cases leaving the eosinophils infiltrated in the lesion.

Thus only certain stages in worm infestation produce an eosinophilia; other stages (particularly in severe infestations) produce eosinopenia, but marked eosinophilic infiltrations can be present in the lungs of sheep concomitant with a normal eosinophil blood count.

Again, in the case of allergy an eosinophilia is expected, although an allergic reaction accompanied by a decrease in blood eosinophils followed by a return to common numbers with no eosinophilia has been seen. (Table II.) Here, presumably, the blood eosinophils infiltrated into the lesion but the stimulus was not strong enough to increase their production.

Relative Eosinophilia.—In Table I it can be seen that the distribution of eosinophils is skewed to the right. This is probably due to some apparently healthy sheep showing an increased eosinophil count due to worm infestation. Whatever the reason may be, the practical value of the term eosinophilia is lost if such counts as 13, 15 and 17 per cent. eosinophils are included as normal in the healthy sheep. Hence the records for six sheep bled each month for two years (see first article) were examined for information on this point, and after comparing them with other records it was considered practical to regard all counts of over 9 per cent. eosinophil cells as connoting a relative eosinophilia.

Eosinopenia.—A smear taken from an apparently healthy sheep may reveal no eosinophils. It could be argued that this was due to a certain stage in a slight worm infestation in which an eosinopenia

was produced, but even if this argument was accepted it would have little value in helping to set up a criterion of eosinopenia for the diagnosis of ill health in the sheep, as for practical purposes sheep slightly infested with worms cannot be excluded from among healthy sheep.

As the absence of eosinophils in one daily sample cannot be accepted as an eosinopenia, the records for healthy sheep from which two samples had been obtained were examined and the results given in Table IV. It can be seen that out of 68 sheep sampled twice, eosinophils were in no case absent on both occasions. Thus the complete absence of eosinophils from two smears taken on different days is unlikely to occur by chance, and can be accepted as defining an eosinopenia, thus giving a useful, and sometimes the only, indication of ill health. It should be noted, however, that for lambs under one month old—where the eosinophils average only 1·1 per cent. (Holman, 1944)—this definition is not reliable.

TABLE IV

PRESENCE OF EOSINOPHILS IN SMEARS FROM HEALTHY SHEEP, SAMPLED ON TWO OCCASIONS

Number of Sheep	*Approx. Interval Between Samples*	*Eosinophils Absent*	
		On One Occasion	*On Both Occasions*
16	1 day	2	0
11	2 days	2	0
15	3 „	3	0
6	Week	1	0
20	Month	5	0
68		13	0

Eosinophils possess some phagocytic powers (Weinberg and Seguin). It should be noted, however, that during an infection with tick-borne fever the bodies which may be found within the cytoplasm of an eosinophil are not necessarily phagocytosed.

Basophils

The occurrence of these cells corresponds to some extent with the eosinophils: they are usually absent during eosinopenia and often present during eosinophilia. An odd basophil may, however, appear at any time, including periods of eosinopenia, and even in some cases a few hours before death. Hence they have little clinical value.

Basophilia.—In a pining lamb a count of 16 per cent. basophils was recorded; *post-mortem* examination did not reveal the cause of ill health. No other cases have been seen but the possibility of their appearance makes it advisable to define the term basophilia. From the data furnished in Table I, this definition would be the presence of more than 3 per cent. basophils.

Total Leucocytes

Leucocytosis.—In man the existence of a physiological leucocytosis is accepted and hence the term does not necessarily connote ill health. In view of this a more arbitrary attitude can be adopted in fixing a useful limit in sheep so that the largest proportion of animals reacting to stimuli can be diagnosed as abnormal. The figure of 13 thousand per c.mm. excludes only three out of 113 apparently healthy animals and would seem from general experience to be a useful standard for leucocytosis.

Leucopenia.—In low leucocyte counts the sampling error is much bigger than in high counts, and it is advisable to scan a larger area. Had this precaution been permissible it is unlikely that the figure of the lowest count, *viz.*, 1·1 thousand, would have appeared. However, by allowing the same latitude as was done in the case of leucocytosis, and excluding the three extreme counts, the figure for leucopenia is set at four thousand, and this is acceptable as a practical standard.

SUMMARY AND CONCLUSIONS

In this article an attempt has been made to set for the sheep certain standards similar to those already well established for man; no new conceptions have been introduced other than the use of the Maximum Admissible Difference.

Typical reactions of different degrees of severity as produced in sheep by pyogenic organisms, are described. They consist of three stages, *viz.*, the neutrophilic invasion phase, the defence phase in which the neutrophilic reaction subsides and the recovery phase indicated by the appearance of numbers of eosinophils. Usually there is a temporary decrease in neutrophils immediately after an injection. The period required for the complete reaction varies and may take three days or three weeks. The neutrophilic reaction can commence as soon as one hour after injection.

Examples of atypical reactions are given: they are neutropenia, agranulocytosis, and ill health indicated by eosinopenia alone.

The following standards are set: *Leucocytosis*—Slight, $13{\cdot}0 \times 10^3$ per c.mm.; marked $20{\cdot}0 \times 10^3$. *Leucopenia*—$4{\cdot}0 \times 10^3$. *Neutrophilic Reaction*—Slight, an increase of 16 per cent. neutrophil cells or of 2 per cent. band forms, between counts on two successive days. Marked, and severe, as for "shift to the left." *Shift to the left*—Slight, 6 per cent. band forms. Marked, metamyelocytes constituting one-third of the neutrophil cells. Severe, metamyelocytes forming one-half of the neutrophil cells. (An exception to the last two definitions occurs when an infection with tick-borne fever is also present.) *Neutrophilia*—Relative, 47 per cent. neutrophil cells: absolute, $5{\cdot}6 \times 10^3$ per c.mm. *Neutropenia*—Relative, 11 per cent.: absolute, $0{\cdot}7 \times 10^3$. *Eosinophilia*—Relative, 8 per cent. eosinophils. *Eosinopenia*—Relative and absolute, absence of eosinophils in 200 cell counts on two smears made on different days.

It is concluded that neutrophils and eosinophils are the two cell-types most helpful as a guide to diagnosis and prognosis, but that many slight reactions will be overlooked unless samples are taken daily.

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[*Received for publication June 24th, 1944*]

BLOOD COPPER STUDIES IN LAMBS IN NORTH WALES

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INTRODUCTION

In 1939 collaboration between the University College of North Wales at Bangor (W. T. Rowlands, Veterinary Investigation Officer) and the Veterinary Laboratory of the Ministry of Agriculture at Weybridge (Dr. H. H. Green as biochemist and Dr. E. L. Taylor as parasitologist) was commenced to investigate a condition of "bad thriving" or "pining" of lambs which was suspected to be due to a combination of specific malnutrition and parasitic infestation. The first year's work reported upon by Rowlands and Harbour (1941) showed that the condition, which was characterised mainly by failure to make adequate live weight gains, sometimes responded to anthelmintic treatment alone, sometimes to oral administration of a "trace element mixture (containing iron, copper, cobalt, manganese and iodine) alone, and sometimes to a combination of both. The experimental work was extended in the ensuing year and, as part of the plan, blood studies were undertaken at Weybridge. These included a series of copper determinations and, following the lines of two previous papers (Eden, 1939, 1941) dealing with blood copper in relation to sheep "pining" in Northumberland, the Welsh data are now presented independently of the principal findings submitted to the Agricultural Research Council.

The significance of blood Cu has been emphasised of recent years in its particular regard to livestock kept in areas where, broadly speaking, a trace element problem has been shown to exist. One such problem is that of "swayback" or "enzootic ataxia" of young lambs. In this country the work of Dunlop and Wells (1938) followed by that of Dunlop, Innes, Shearer and Wells (1939) showed that this condition can be controlled by feeding supplementary Cu to the pregnant ewe, and associated blood Cu studies have demonstrated the low Cu status of ewes in areas where "swayback" is endemic. The Cu status of the herbage, however, appeared to be adequate and values of 12 to 24 p.p.m. on a dry matter basis were considerably higher than values of 7 to 10 p.p.m. reported (Eden, 1939) in areas of Northumberland where "swayback" is unknown.

The enzootic ataxia of Western Australia can also be prevented by feeding Cu supplements to the pregnant ewe, but the disease, on the other hand, appears to be correlated with a very low Cu status both of the animal and of the herbage (less than 5 p.p.m.). Pathologically, "swayback" and enzootic ataxia are identical conditions. The main difference lies in the fact that in this country a low Cu status of the animal is not correlated with a low Cu content of the herbage.

PRESENT INVESTIGATION

In the present paper it is unnecessary to give full protocols of the experiment. It is sufficient to say that the lambs on each farm were divided into four groups, namely, those receiving (*a*) anthelmintic, (*b*) "trace element mixture," (*c*) combined anthelmintic and trace element treatments, and (*d*) controls. On Farm A, which was Centre No. 2 of the previous year's experiments, a fifth group receiving cobalt was included. For the purposes of the present paper (*b*) and (*c*) can be combined as one Cu-treated group and the remainder as a group without Cu. The amount of Cu supplied was 42 mg. as the sulphate, in solution each fortnight. The lambs were either pure Welsh or Welsh x Cheviot-Border Leicester crosses, born in March and April. Treatment commenced at the end of May and continued until the lamb sales in September. During July, samples were taken of the soil and vegetation on each farm and, besides the usual agricultural analyses, were examined specifically for Fe and Cu.

On Farm A bleedings of all lambs were carried out on three occasions, namely, on May 31st, July 7th, and on September 4th. Blood was withdrawn by jugular puncture into Cu-free potassium oxalate as anticoagulant, and analysed for haemoglobin by the acid-haematin method and for Cu by the diethyldithiocarbamate method (Eden and Green, 1940). Some blood samples were also allowed to clot for serum; on these certain other minerals were estimated, but as all the figures lay within the normal range they are not reported here. The haemoglobin data presented no abnormal features and they are also omitted from the present text.

Similar serial studies were carried out on five other farms in the North Wales area although in somewhat less detail than on Farm A where there was preliminary evidence of the beneficial effects of trace element therapy. Since it was found that the data fell into two distinct classes, those on Farm A and those on the other five farms, the latter are grouped together irrespective of the dates on which the samples were taken, and will be referred to as Farm B samples.

"Swayback" in lambs was not unknown on Farm A although very few cases had occurred during the present season. No reports of swayback had been received from any of the B group of farms.

The main analytical data are summarised in Table I.

When all the observations on Farm A are compared with those from Farms B it will be seen that the average blood Cu of the former was only slightly more than half that of the latter. At the extreme upper limits the difference was not well marked, but at the lower limits it was very striking. No value on the B farms fell below 0·06 mg.p.c. whereas the average value of the lambs on A was initially less than 0·05 mg.p.c. This figure rose to 0·07 mg.p.c. in July but fell again in September to 0·06 mg.p.c. Of the groups receiving supplementary Cu as compared with non-Cu treated groups, there were no differences in the B Farms. The non-Cu groups on Farm A rose slightly from

0·047 to 0·06 in July, but fell to practically the initial value in September (0·050). It may be stated without giving the full protocols that the occurrence of the bad-thriving condition in lambs was not directly correlated with specific low blood Cu levels.

The distribution of the individual values are shown by a series of histograms given in Figure I plotted on a percentage basis to bring the varying numbers to a common denominator. Charts (*a*) and (*b*) show the distribution, at intervals of 0·02 mg. Cu per 100 c.c., of all the data in Farm A and Farms B respectively. Values below 0·06 and above 0·12 mg.p.c. are shaded, and inspection readily shows the decided "shift to the left" of the Farm A values; 57 per cent. of the observations fall below 0·06 in Farm A, as compared with none in Farms B, whilst of the latter over 20 per cent. exceed 0·12 mg.p.c. as

TABLE I

BLOOD COPPER VALUES IN LAMBS

	Farms.	Number of Samples	Mean Blood Cu mg. per cent.	Range mg. per cent.
1. A	May 31st ...	85	0·047	0·01 to 0·13
2. A	July 7th	89	0·070	0·01 to 0·17
	(*a*) Cu-treated	37	0·075	0·02 to 0·13
	(*b*) Non Cu-treated	52	0·066	0·1 to 0·17
3. A	Sept. 4th ...	97	0·060	0·01 to 0·13
	(*a*) Cu-treated	39	0·074	0·03 to 0·13
	(*b*) Non Cu-treated	58	0·050	0·01 to 0·12
4. A	All observations	271	0·059	0·01 to 0·17
5. B	... ...	226	0·105	0·06 to 0·19

compared with about 2·5 per cent. of the A values. The Farm A values are more scattered around the mean than those of Farms B.

Chart (c) shows the distribution of the Farm A data at the end of May, *i.e.*, before any treatment was commenced, while Charts (*d*) and (*e*) respectively, show the distribution of Farm A values in July and September. In Chart (*c*) nearly 78 per cent. of the values lay below 0·06 mg.p.c. In July this figure was about 37 per cent., and in September 57 per cent. Few values exceeded 0·12 mg.p.c.—in May 1 per cent., in July 5 per cent., and in September 2 per cent. From Charts (*d*) and (*e*) it will be seen that those groups on Farm A which were receiving Cu treatment showed essentially the same type of distribution as the groups which were not receiving Cu treatment except for a slight displacement to the right in the former. The effect of Cu treatment in July was shown by a slight rise to a mean of 0·075 mg.p.c. as compared with 0·066 mg.p.c. means of the groups without Cu; and in September of 0·074 compared with 0·050 mg.p.c.

In Australia, where low blood Cu values have been reported in association with definite pathological conditions in sheep, lambs and cattle, there has been found a correspondingly low Cu status of the herbage. Beck (1941) in a survey of the pastures of areas where diseases accompanied by low Cu status of the animal are prevalent

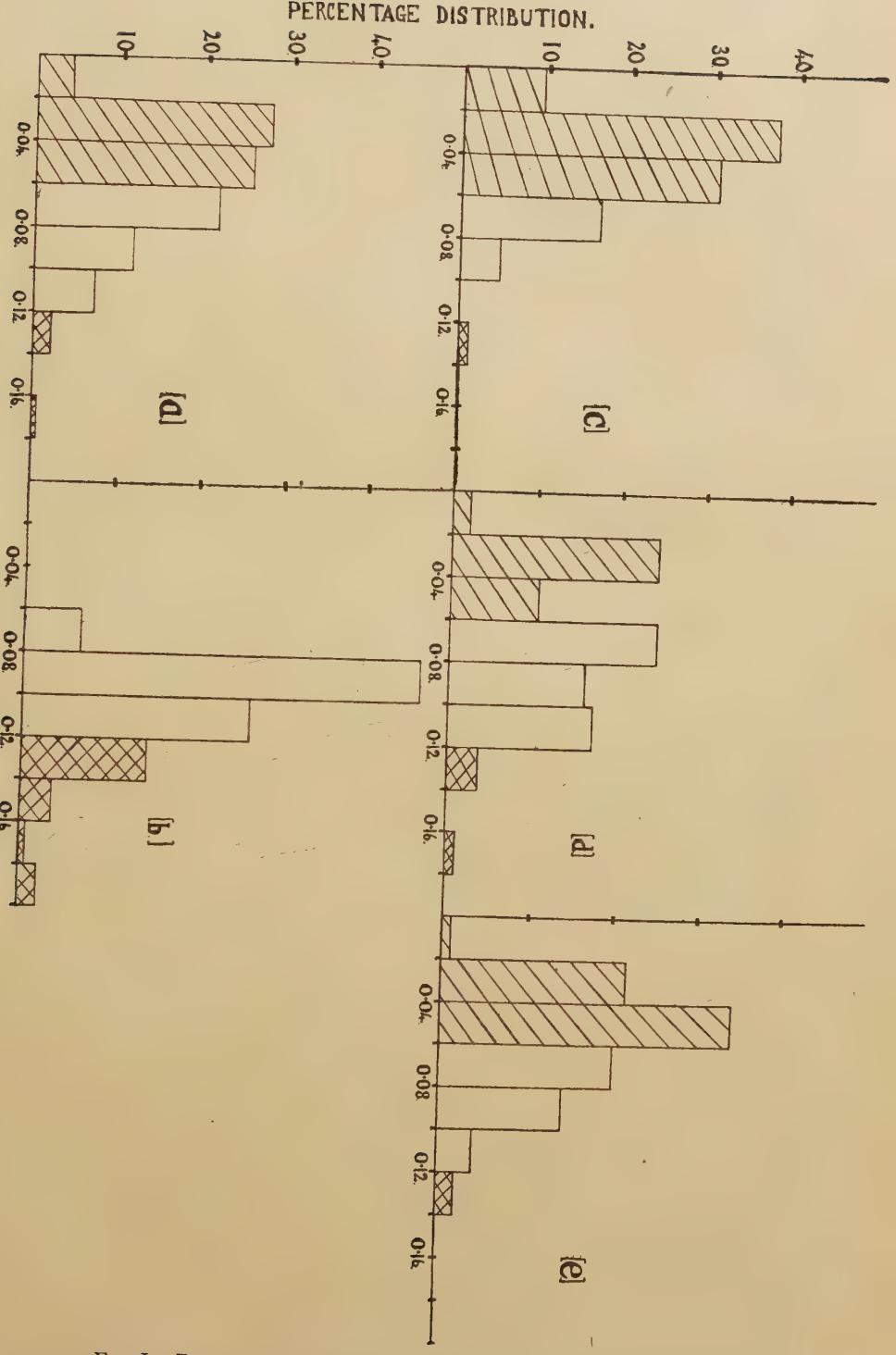

FIG. I. Percentage Distribution of Blood Copper Values in Sheep.
Chart (a) Distribution of all observations on Farm A; Chart (b) Distribution of all observations on Farms B; Chart (c) Distribution of Farm A values in May; Chart (d) The same in July; Chart (e) The same in September.

was able to conclude that "sound" areas contained more than five parts of Cu per million of dry matter and "unsound" areas less than 3 p.p.m. This seemingly close connexion between pasture and blood Cu levels in Australia has not been borne out by any investigator in Britain. A number of soils and pastures were analysed in the present investigation and the results are reported in Table II.

It will be noted that the soils on Farm A had the highest Cu content. One of Farms B (No. 3) showed a very low value of 3 p.p.m. This was a farm on the seashore and parts of it are frequently washed over by the sea. Nevertheless the herbage contained 12 p.p.m. Cu The figures for the soil and herbage on Farm A show no evidence of deficiency when they are compared with those from the Farms B or when compared with those from Australia. In Holland pastures containing 10 p.p.m. are regarded as adequate in Cu status (Sjollema, 1938). Eden (1939) reported figures of 7 to 10 p.p.m. from the

TABLE II

COPPER ANALYSES OF SOIL AND PASTURE: JULY, 1941

Farm A.		*Farms B.*	
Soil Cu p.p.m. (Air-dry basis)	*Pasture Cu p.p.m. (Dry matter basis)*	*Soil Cu p.p.m. (Air-dry basis)*	*Pasture Cu p.p.m. (Dry matter basis)*
1. 35	1. 13	1. 20	1. 27
2. 32	2. 21	2. 21	2. 18
3. 40		3. 3	3. 12
4. 32		4. 24	4. 16
5. 36		5. 27	5. 29

Northumberland area. The present evidence therefore indicates that Farm A has adequate total Cu in the soil and pastures, and that it is not possible to relate the low blood Cu levels of sheep grazing on this farm to the absolute Cu content of the herbage. Whatever may be the cause of these low levels in the sheep, it does not appear to be due to Cu deficiency as such. This is further borne out by the observations that periodic dosing of the lambs on Farm A with a mineral mixture containing, *inter alia,* 42 mg. Cu (= to about 0·17 g. $CuSO_4.5H_2O$) every fortnight did not affect very materially the general blood Cu level and certainly did not raise the average to that found in the lambs on Farms B.

A previous survey of blood Cu distribution in the Northumberland area (Eden, 1939) had shown that ewes of ages varying from one to six years had an average level of blood Cu of 0·09 mg.p.c., ranging 0·02 to 0·16 mg.p.c. Although regular monthly dosing with 100 c.c. of 1 per cent. copper sulphate solution raised the average from 0·07 to 0·10 mg.p.c., the feeding of a heavy mineralised cake containing Cu sufficient to double the normal grazing intake had only a slight effect, and some of the lowest figures in the various groups were found in ewes which were receiving additional Cu in the food. The

conclusion was reached that the wide variations in blood Cu values among individual sheep were due to a natural phenomenon other than Cu deficiency as such.

Innes and Shearer (1940) in an investigation of the "swayback" areas of Derbyshire reported low blood Cu values amongst ewes giving rise to "swayback" lambs, and controlled the disease by feeding extra Cu to the pregnant ewe. In a later paper Shearer, Innes and McDougall (1940) confirmed the earlier observations on the low Cu status of lambs suffering from "swayback" and showed that the pastures even in the winter contained 8 to 18 p.p.m. Cu. These authors suggested that there might be some factor in the grass interfering with the proper assimilation of Cu.

Hence, as far as this country is concerned there is now evidence from widely separated areas of a low Cu status in sheep not connected with corresponding low copper values in the herbage. Those areas are not deficient in the same sense as the Cu deficient areas in other parts of the world.

In connexion with the present studies Farm A seems to be somewhat anomalous. "Pining" or "bad thriving" has been known on it for some years, and preliminary evidence has already shown that the condition responds to trace element therapy in general and to cobalt in particular. During the season in which the present studies were made this "bad thriving" condition was not so manifest as in previous years although the general effects of trace element therapy were reflected in the increased live weight gains in the treated groups. Since this work was carried out there has been additional evidence from other areas that cobalt may play a part in increasing live weight gains of lambs. The exact physiological role of both Co and Cu in the animal body awaits more detailed investigation, and at the moment little is known about the interrelationships of the trace elements. On Farm A the facts are that there is a low Cu status in animals grazing on pastures which are of adequate Cu content, and that on this farm lambs can show a remarkable response to trace element therapy. As a tentative hypothesis it may be suggested that there is possibly some factor in the herbage, unknown at present, which in some way interferes with the assimilation or physiological utilisation of copper (and possibly cobalt) with consequent failure of the lambs to attain normal development. There is no evidence at the moment for the actual existence of such a factor or of its nature if it exists, but in this connexion the following supplementary experiment may be of interest.

Eight lambs whose live weights at the end of May lay between 32 and 42 lb. grew normally until the beginning of August, when they either made no further gains or actually lost weight over the ensuing three months. They were purchased and brought to Weybridge in the middle of November and fed on concentrates and hay. Growth immediately recommenced and they gained steadily until the following April. The blood copper data are given in Table III.

The November bleedings were made immediately the lambs arrived at Weybridge, two days after they had been purchased at Farm A. Lamb No. 1 died from pneumonia early in February, and its figures are excluded from the general means. Analyses of the food supplied at Weybridge indicated that the Cu intake did not exceed that which the lambs had received from the natural pasture (based on the July analyses), and the general increase in blood Cu level is therefore of special interest. Apart from Lamb No. 4 at the November bleeding no value fell below 0·06 mg.p.c., whereas of the eight original lambs five in May, three in July and five in September had values below this figure.

The data for the lambs on the Weybridge diet fit in with the more commonly accepted conception of normal blood Cu levels and are of the same order as those of Farms B as reported in the earlier part of this paper. It is perhaps unwise to infer very much from

TABLE III

BLOOD COPPER DATA ON PINING LAMBS BROUGHT TO WEYBRIDGE.
CU IN MG. PER CENT.

Number	*On Farm A*			*At Weybridge*					
	May	*July*	*Sept.*	*Nov.*	*Dec.*	*Jan.*	*Feb.*	*Mar.*	*Apr.*
1.	0·05	0·03	0·03	0·06	0·09	0·10	—	—	—
2.	0·02	0·03	0·04	0·08	0·11	0·15	0·09	0·09	0·14
3.	0·03	0·09	0·12	0·11	0·11	0·13	0·12	0·12	0·12
4.	0·02	0·03	0·04	0·04	0·12	0·17	0·08	0·10	0·12
5.	0·08	0·08	0·06	0·10	0·11	0·13	0·10	0·09	0·11
6.	0·08	0·12	0·09	0·07	0·11	0·12	0·11	0·09	0·12
7.	0·06	0·06	0·03	0·07	0·10	0·12	0·10	0·09	0·11
8.	0·03	0·09	0·03	0·08	0·13	0·12	0·10	0·10	0·18
Mean excluding No. 1	0·05	0·07	0·06	0·08	0·11	0·13	0·10	0·10	0·13

these somewhat restricted data, but the fact that the blood Cu levels of the lambs after removal from the pastures on Farm A quickly rose to the Farms B levels is not in conflict with the hypothesis that a Cu-depressing factor exists in the pastures of this farm. Further experimental work is necessary before such a hypothesis can be substantiated.

SUMMARY

Blood Cu studies were carried out on the lambs of six different farms in North Wales where conditions of bad thriving or pining were being investigated. On five of these farms the lambs showed a normal blood Cu content, the average of 226 samples being 0·105 mg.p.c. On the sixth farm the lambs showed a much lower average, and a series of 271 observations from May to September gave an average of less than 0·06 mg.p.c., with extreme values as low as 0·01 mg.p.c. On this particular farm bad thriving, responding to trace element—and in particular, cobalt—therapy, had been well marked, although the soil and pasture had shown an adequate Cu

status. When affected animals with low blood Cu values were transferred to Weybridge, the figures rose to normal levels within a comparatively short time, although the absolute Cu intake had not been appreciably increased. A tentative suggestion of a Cu-depressing factor in the natural herbage is put forward.

ACKNOWLEDGMENTS

Thanks are due to Dr. H. H. Green and to Mr. W. T. Rowlands, M.R.C.V.S., D.V.S.M., for the opportunity of participating in the experimental work on bad thriving in lambs in North Wales.

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[*Received for publication July 10th, 1944*]

ACTINOBACILLOSIS IN SHEEP

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INTRODUCTION

Although actinobacillosis is recognised as one of the more common diseases of cattle, and the causal organism is known also to infect sheep, the literature contains few references to the disease in sheep. Thus, outbreaks, mainly isolated and affecting usually only a few animals have been recorded in England (Bosworth, 1927), in South Africa (Thomas, 1931), and in Italy (Ravaglia, 1934). More recently, Marsh and Wilkins (1939) and Davies and Styles (1939) in North America, and Wramby (1940) in Sweden have also described its occurrence.

A disease of sheep, characterised by chronic abscess formation in the tissues of the head and in the lungs, was recorded by Christiansen (1917) in Denmark, who isolated and described the causal organism as *B. purifaciens*. This condition was re-described in some detail by Magnusson (1929) in Sweden. The organism was identified by Jowett (1931) as a cause of pyaemia in sheep lungs obtained from an abattoir in Scotland. A similar disease, appearing to affect mainly rams, has long been known in Scotland, where it is widespread in its distribution, and was indeed recognised as a clinical entity by Russell Greig in 1920.

That the above infections reported as caused by *B. purifaciens* and actinobacillosis are one and the same pathological entity is indicated by evidence recently accumulated. That they are clinically indistinguishable was first suggested by Bosworth and Glover (1931) who, in referring to *B. purifaciens*, stated that "another agent responsible for a very similar clinical picture is the actinobacillus. We have noted its presence in various parts of the head and associated glands, in the lungs and thoracic glands." Marsh and Wilkins (1939) suggested that the two diseases were identical, while Tunnicliff (1941), who studied the strains of *A. lignieresi* isolated by them and compared her results with the published description of *B. purifaciens* (a strain of which was not available to her), concluded that they were the same organism. Wramby (1940) also stated that the two organisms were identical.

The purpose of this paper is to confirm the identity of *A. lignieresi* with *B. purifaciens* and to record the characteristics and widespread occurrence in Scotland of actinobacillosis in sheep.

BACTERIOLOGICAL EXAMINATION

Source of strains.—Three strains of *A. lignieresi*, Nos. 4189 and 4190 of the National Collection of Type Cultures, and No. 115, recently isolated from a bovine lymph node by Professor T. J. Bosworth, and seven strains of *B. purifaciens* were available for comparison. The latter were isolated from clinical cases of the disease in sheep, Nos. 4892 and 4929 in ewes, No. 4955 in a lamb, and Nos. 4951, 4956, 4964 and 4966 in rams.

Cultural characteristics.—The morphology and the behaviour of *A. lignieresi* on artificial media are well known and agree essentially with the various published descriptions of *B. purifaciens.* In brief, both organisms are small, gram negative bacilli, facultatively anaerobic, varying in morphology with the medium and time of incubation. Both multiply rapidly on solid media on which they show no special characteristics other than a variable degree of adhesion to the medium and a faint bluish coloration which is best demonstrated by transmitted light. Growth in liquid media is apparently improved by the addition of serum, and a surface pellicle, which disintegrates readily may or may not form. Both organisms die out rapidly unless they are subcultivated every few days, although as was noted by Bosworth (1923), broth cultures survive rather longer, namely, up to ten or twelve days.

Stab cultures of *A. lignieresi* in deep agar are said to give a whitish spot of growth on the surface (Topley and Wilson, 1936), but no growth down the needle track, whereas both Magnusson (1929) and Jowett (1931) describe *B. purifaciens* as forming a horse shoe nail growth. All our strains of both organisms behave identically in this medium, giving a heavy growth on the surface and a filiform growth down the line of inoculation.

Biochemical reactions and fermentation of sugars.—Topley and Wilson (1936) describe the fermentative ability of *A. lignieresi* as being in some doubt, and recently Slavin (1944) has described an organism isolated from cattle, possibly a variant of the classical type, which was incapable of fermenting any of the usual sugars. Lemco broth with 1·0 per cent. of the sugar to be tested and Andrade's indicator was used in our tests. Each strain was tested on at least three occasions, the results being read after ten days' incubation. On each occasion the findings were in correspondence, except in the case of arabinose, raffinose, and glycerol which were fermented irregularly. The ten strains each showed the characteristic late fermentation of lactose. The results are shown in Table I which also compares the findings of previous authors.

Litmus milk remained unchanged and indol was not produced.

Pathogenicity for laboratory animals.—Two guinea-pigs were inoculated, one subcutaneously and the other intraperitoneally, with 1·0 c.c. of each strain grown overnight on agar and standardised in saline to No. 9 on Brown's scale. No ill effects were observed and at autopsy five weeks later no lesions were demonstrable. A similar dose of organisms was without effect when inoculated subcutaneously into rabbits, although intravenous inoculation of the more recently isolated strains was followed by the death of the animal within eighteen hours. Smaller doses were slightly toxic, but they were incapable of stimulating the production of visible lesions.

Serological tests.—Both Thompson (1933) and Tunnicliff (1941) have described an irregular cross-agglutinability among different strains of *A. lignieresi,* but eleven strains which had been isolated by

TABLE I

Sugar	A. lignieresi (3 *strains*)	B. purificiens (7 *strains*)	*Magnusson* (1929)	*Jowett* (1931)	*Bosworth* (1923)	*Davies and Torrance* (1930)	*Tunnicliff* (1941)	*Wramby* (1940)
Saccharose ...	+	+	+	+	+	+	+	+
Lactose ...	+	+	+	+	+	+	+	+
Laevulose ...	+	+	+		+	+	+	+
Glucose ...	+	+	+	+	+	+	V	+
Galactose ...	+	+			+	+	+	+
Raffinose ...	V	V	—				—	—
Rhamnose ...	—	—	—				—	—
Maltose ...	+	+	+	+	+	+	+	+
Mannose ...	+	+	+				+	+
Mannite ...	+	+	+	+		+	+	+
Inulin	—	—	—			—		—
Xylose	+	+	+			+	+	+
Salicin	—	—	—	—		—	—	—
Sorbite	—	—	—			—	—	—
Adonite ...	—	—	—	—	—			—
Erythrite ...	—	—	—		—			
Arabinose ...	V	V	—				—	—
Dextrin ...	+	+					—	+
Inosite	—	—	—					—
Glycerol ...	V	V	—				V	+
Trehalose ...	—	—					—	—

+ = Fermented. — = Not fermented. V = Irregularly fermented.

TABLE II

Serum	*Antigen*									
	4189	4190	115	4892	4929	4951	4955	4956	4964	4966
4189	1/2560	1/10	1/10	N	1/320	1/320	1/320	1/640	1/160	1/80
4190	1/40	1/2560	N	1/640	1/640	1/1280	1/640	1/160	1/40	1/20
4892	1/40	1/80	1/1280	1/5120	1/640	1/2560	1/2560	1/2560	1/640	1/640
4929	1/80	1/160	1/5120	1/1280	1/5120	1/2560	1/10240	1/5120	1/5120	1/320
4951	1/160	N	1/2560	1/320	1/2560	1/10240	1/10240	1/10240	1/160	1/640
4955	1/20	N	1/10240	1/640	1/5120	1/10240	1/10240	1/10240	1/2560	1/10240
4956	1/20	N	1/2560	1/320	1/1280	1/5120	1/10240	1/10240	1/640	1/1280
4964	N	1/10	1/640	1/320	1/2560	1/640	1/1280	1/1280	1/5120	1/1280
4966	1/80	N	1/10240	1/5120	1/5120	1/10240	1/10240	1/10240	1/2560	1/10240

N = Negative in a dilution of 1/10. Highest dilution tested = 1/10240.

Davies and Torrance (1930) were all agglutinated to the same titre by a serum prepared against one of them.

Agglutinating sera against our strains were prepared from rabbits; four weekly intravenous inoculations of living organisms, 0·5 c.c. of an overnight culture on agar, standardised to No. 5 on Brown's scale, were given to each animal, which was bled seven days after the last injection. Some difficulty was encountered in the preparation of antigen, particularly from those strains which adhered to the medium. Eventually overnight growths on Hartley's agar were suspended in normal saline, shaken for 30 to 40 minutes in a mechanical shaker, filtered through eight layers of butter muslin and standardised to No. 4 on Brown's scale. The tubes were incubated for eighteen hours at 37° C. and the highest dilution which caused complete agglutination was accepted as the titre of the serum. The result of cross-agglutination tests is shown in Table II.

The serum of clinically affected animals commonly agglutinated the homologous antigen in a dilution of 1 : 80 to 1 : 320; heterologous antigens were agglutinated usually, but not always, by lower dilutions. Samples of blood serum from 14 sheep, which were presumed to be either normal or were suffering from conditions other than actinobacillosis, were also tested against each strain. Every serum except one agglutinated several of the antigens in dilutions of 1 : 20 to 1 : 40 and conversely each antigen reacted with the majority of the sera. The exception proved to be that derived from 4190, which was not agglutinated by any of the serum samples. A result similar to this was obtained by Tunnicliff (1941) with normal sheep serum.

Tests on absorption of agglutinins were performed with four sera. Each serum was absorbed in a dilution of 1 : 20 with a heavy suspension of a heterologous antigen until the antigen was no longer agglutinated. Agglutination tests with these sera are recorded in Table III.

TABLE III

Serum	*Absorbed by*	*Antigen*								
		4189	4190	115	4892	4929	4951	4955	4956	4964
4189	4956	1/640	N	N	N	N	N	N	N	N
4190	4951	N	1/640	N	1/80	1/160	N	N	N	N
4892	4955	N	N	1/320	1/1280	N	N	N	N	N
4929	4964	N	N	N	N	N	N	N	N	N

N = Negative in a dilution of 1/80.

The results of cross-agglutination and agglutinin absorption tests confirm the observations of Thompson (1933) and Tunnicliff (1941) that there may be considerable antigenic differences among strains of *A. lignieresi*. This may vary, however, with the length of time that the organisms have been in artificial culture: the relationship between the recently isolated strains of *B. purifaciens* and *A. lignieresi* 115 would appear to be comparatively close.

It may be concluded that *B. purifaciens* and *A. lignieresi* are culturally and biochemically identical, and that although they are close, the serological relationships of strains of both organisms may be subject to individual variation. In the remainder of this paper, therefore, the term *B. purifaciens* will be discarded in favour of the older designation.

DISTRIBUTION, INCIDENCE, ETC., OF ACTINOBACILLOSIS

In the course of routine diagnosis during 1942 and 1943, cases of actinobacillosis in sheep have been encountered in the counties of Moray, Aberdeen, Perth, Berwick, Roxburgh and Dumfries, while the disease is known to veterinary practitioners in Argyll, Peebles and elsewhere. That it is commonly encountered in the Edinburgh abattoir has been recorded by Jowett (1931).

The condition seems to be well known within the agricultural community as "cruels" or "king's evil" but, as the incidence in any one flock is commonly low, advice is but rarely sought, and the majority of cases would appear to pass unrecognised.

It is commonly stated that no previous history of the disease on the farm can be obtained. The truth of this statement is sometimes doubtful, for on certain farms it is recognised year after year, and on one farm in particular a number of the rams have been affected annually for at least twenty years. The majority of the cases examined have occurred in Scottish Blackface sheep but the disease has been seen in other breeds, and there is no reason to doubt that all are equally susceptible. To many clinicians the condition is known to occur only in adult rams, but lambs five to six months of age and ewes may also be affected; the last, particularly under conditions of hill management, are often not under particularly close supervision, and consequently the disease in them, especially in the early stages, may be unsuspected or even disregarded.

The actual incidence of infection throughout any particular flock is difficult if not impossible to ascertain with any degree of accuracy, but on one farm, where only the rams appeared to be infected, seven of 35 showed clinical symptoms of the disease. Davis and Styles (1939) found six rams of 80 to be affected, while Marsh and Wilkins (1939) recorded about 60 deaths from the disease in a flock of 2,000 ewes.

Infection.—It is usually believed that the actinobacillus gains entrance to the tissues of cattle by the infection of wounds or abrasions, particularly of the mucous membrane of the mouth, and it has been stated (Thornton, 1943) that "on the Continent numerous cases of actinobacillosis, almost reaching the proportions of an epidemic have occurred after attacks of foot-and-mouth disease." That the route is similar in sheep was suggested by Thomas (1931), who attributed infection to the eating of prickly pear, but found that the disease disappeared when the diet was improved.

In a number of the cases examined, the portal of entry of the

organism was not obvious, but in three instances wound infection could be implicated. (1) Blackface rams, kept throughout most of the year on a pasture surrounded by a wire fence had developed the habit, common to all animals, of putting their heads between the wires in order to eat the grass on the other side. It sometimes happens that the head gets caught in the fence, the wire passing through the curve of one horn, under the throat and through the other horn, thus permitting lateral but no antero-posterior movement. An animal may be fixed thus for several hours before it is seen and released, by which time the skin at the angle of the jaw may be considerably damaged. The primary lesions of the disease in these sheep appeared at the site of the skin abrasions. (2) Lesions of actinobacillosis appeared simultaneously in a number of young animals shortly after the administration of anthelmintics by means of a balling gun. (3) The disease was observed in three five to seven months' old lambs among a number of Blackface sheep purchased for the preparation of louping-ill vaccine. In each of these the primary lesion was in the skin covering the frontal bone at the base of the horn, *i.e.*, where bruising results from fighting.

Clinical symptoms.—Sheep in the early stages of the infection may show little change other than enlargement of the lymph nodes of the head, in particular of the submaxillary. The primary lesion in the lambs mentioned in (3) above took the form of a shallow ulcer in the skin at the base of the horn which discharged the typical pus associated with the disease. Later, the submaxillary lymph node on the same side showed enlargement and eventually suppuration.

The disease in its later stages has been adequately described by earlier workers. Numerous abscesses reaching in size that of a small orange form in the tissues of the head and its associated lymph nodes. The skin and subcutaneous tissues become thickened and discharged pus forms crusts in the wool of the face and neck. The prescapular lymph nodes may become greatly enlarged and readily palpable. No marked loss in condition appears until the stage is reached when prehension and mastication of food and respiration become hampered by abscess formation.

Post-mortem appearance.—The characteristic lesion is a thick-walled abscess containing a peculiarly viscid greenish yellow pus. In the majority of cases the pus is of an even consistency, as was noted by Ravaglia (1934), while in others it may contain a number of pale yellowish-white non-calcified "granules" which disintegrate readily on manipulation.

In advanced cases, the skin and subcutaneous tissues of the head, particularly in the region of the face, are thickened and contain numerous abscesses. Increased production of fibrous tissue does not appear to be as characteristic a feature of the disease in sheep as it is in cattle. In this commonly affected region of the body it can be ascribed to the continual formation and rupture of fresh lesions, the pus from which, however, is seldom if ever completely discharged.

The demonstration of normal lymphatic tissue in the head may prove difficult, the lymph nodes being almost totally replaced by abscesses of varying size and reaching that of a small orange. On no occasion have lesions been found in the tongue, an unexpected observation in view of the comparative frequency with which this organ is affected in cattle. Occasionally abscesses may be found in the body when no abnormality can be detected in the head region. Thus, the organism was isolated by us from the prescapular lymph node of a sheep which was killed during the preparation of louping-ill vaccine and which showed no other lesions. Bosworth and Glover (1931) observed actinobacillosis in a carcase gland of a sheep in the course of meat inspection. Although lesions have been described as occurring in the liver, kidneys and elsewhere, their commonest sites apart from the head are the lungs and prescapular lymph nodes, which may contain up to 100 c.c. of pus. The lesions in the lung are found scattered throughout its substance and may reach 3 cm. in diameter. On section, they discharge the typical pus.

A. lignieresi may sometimes be associated with lesions affecting the bones of the head of cattle. In advanced cases of the disease in sheep marked changes may also be present in the head bones. The mandible shown in Fig. I was obtained from an aged Blackface ewe in which the disease was advanced. In earlier cases exostoses may be present.

Histological appearance.—The microscopic appearance of the lesions of bovine actinobacillosis are well recognised and may be described as being essentially fibrotic. In sheep they differ in that suppuration is much more marked and the lesions appear to consist of abscesses of varying size, each enclosed in a fibrous capsule. Chronic inflammatory changes may also be evident. In the abscesses, granules may be found scattered throughout the pus but they are present in greatest number at the periphery of the lesion. Giant cell formation has not been observed. In the lungs, the lesion is a septic embolic consolidation with occasional rupture of the air passages and the appearance of pus in the bronchioles. It was at first thought that the frequent occurrence of abscesses in the lungs might conceivably be due to the inhalation of organisms from foci in the throat, *i.e.*, a true broncho-pneumonia. The concurrent round-cell *infiltration* outside the muscular coat of the bronchioles and normal appearance of the lining, even when pus cells are seen on it, and the oedematous state of the blood vessels, indicate, however, that the lesions originate from infection derived from the blood or lymph, streams (Fig. II).

Experimental reproduction.—The virulence of individual strains of *A. lignieresi* is notoriously variable. The typical disease, as is known, cannot invariably be reproduced in cattle by inoculation. Similar results have been experienced with sheep (Magnusson, 1929; Jowett, 1931).

Several attempts were made to reproduce the disease in sheep with three freshly isolated strains, namely, 4929 and 4951 of ovine

origin and 115 isolated from a bovine lymph node. Blackface lambs six to nine months of age were used. The inoculum was grown overnight on Hartley's agar and standardised in saline to the requisite opacity on Brown's scale.

With the object of producing both local and generalised lesions strain 40929 was inoculated simultaneously in doses of 0·5 c.c. subcutaneously on one side of the face, 0·1 c.c. intradermally on the other side, and 2·0 c.c. intravenously, into each of six animals. Nos. 632 and 634 received a suspension equivalent to No. 9 on Brown's scale, Nos. 635 and 636 received No. 2 on Brown's scale, and Nos. 637 and 638 No. 2 diluted 10^{-1}. Nos. 632, 634, and 636 were found dead on the day following inoculation. At autopsy, the results of which varied only slightly in each animal, thick fluid exuded from the nostrils, the skin was flushed and there was marked congestion of the subcutaneous vessels. The spleen showed petechial haemorrhages, the kidneys were congested but firm, and a considerable excess of blood stained peritoneal fluid was present. In the thorax there was a great excess of pleural fluid, there were subendocardial haemorrhages in the left ventricle, and the lungs were markedly oedematous with patches of congestion. *A. lignieresi* was isolated from the heart blood of 634 and 636. No. 635 showed no abnormality until five days after inoculation, when typical abscesses appeared on the face at the site of the inoculations. These retrogressed and four weeks later had disappeared, but the animal was extremely lame with pus formation in the fetlock joints, one of which discharged the characteristic pus. The subsequent history of this animal is described below under "Treatment." Nos. 637 and 638 both developed abscesses at the inoculation sites on the face, and in four weeks there was some apparent enlargement of the regional lymph nodes; later the abscesses retrogressed and eventually disappeared. No. 637 was destroyed nine weeks after inoculation. The only lesions found, from each of which *A. lignieresi* was isolated, were two small abscesses in one lung and another in the liver. Thirteen weeks after inoculation No. 638 appeared normal and at autopsy no lesions were found.

An attempt was made to infect six sheep by scarification. The wool in the throat region was clipped as short as possible, the skin scarified, and a large loopful of strain 4951 applied. The wounds healed rapidly and the autopsy of each animal, six weeks after infection, was negative.

Strain 115 was standardised as before to No. 2 on Brown's scale diluted 10^{-1} and inoculated subcutaneously on the side of the face of No. 729 in a dose of 0·5 c.c. The animal remained normal. A similar inoculum, comparable to No. 9 on Brown's scale was given to No. 727. An abscess developed within seven days and discharged characteristic pus two weeks after inoculation.

Treatment.—Sodium or potassium iodide therapy is recognised as specific in the treatment of actinobacillosis in cattle; its effect was therefore observed on two available sheep, one naturally, and one, No. 635 above, artificially infected. Three injections of 3·0 g. of

sodium iodide in 25 c.c. distilled water were given intravenously at weekly intervals to No. 635. The joint abscesses healed without rupturing and at autopsy ten days after the last injection no lesions were found beyond a slight non-purulent enlargement of one fetlock joint.

A six months old Blackface lamb with an open lesion at the base of one horn and marked enlargement with commencing abscess formation in the submaxillary lymph node of the same side was unsuccessfully treated by similar intravenous injections. The lesions decreased somewhat in size but the response was not sustained and within three weeks of the last injection had resumed their former size; 5 g. of potassium iodide in 30 c.c. distilled water were given by mouth daily for ten days, by which time the lesions had disappeared except for a single purulent focus approximately 1 mm. in diameter in the open lesion. This again progressed but was completely resolved by a second similar course of potassium iodide.

Two veterinary practitioners have reported good results from the oral administration of potassium iodide in the treatment of the disease in the field, particularly when combined with surgical evacuation of the superficial lesions.

DISCUSSION

In the past, several factors have militated against the recognition of infection with *B. purifaciens* as actinobacillosis. Firstly, although the disease is widespread in Scotland and is well known by various local names to the agricultural community, it has a low incidence in individual flocks and many cases are sporadic in origin. Commonly, therefore, little notice is taken of clinical cases, and it is only when valuable animals become affected, or cases numerous, that advice is sought. Secondly, the condition is clinically distinct from that seen in the bovine and is apt to be regarded as "pyaemia." Following the work of Tunnicliff (1941), Wramby (1940), and that described in Part I of this paper, however, the identity of the two organisms may be regarded as established; consequently, the designation *B. purifaciens* should be discarded in favour of *A. lignieresi.*

On the basis of observations on the agglutinin content of normal sheep sera, Tunnicliff (1941) considered it probable that *A. lignieresi* was more widely distributed than is normally supposed. Throughout this work the impression has been gained that the organism may well be a commensal of the mouth and/or skin of sheep in this country, gaining access to the tissues and giving rise to progressive disease only in the presence of as yet unknown factors of which physical injury is probably one.

SUMMARY

1. *B. purifaciens* is identical with *A. lignieresi* culturally, biochemically, and in its effect when inoculated into laboratory animals

FIG. I. Mandible of aged ewe affected with actinobacillosis.

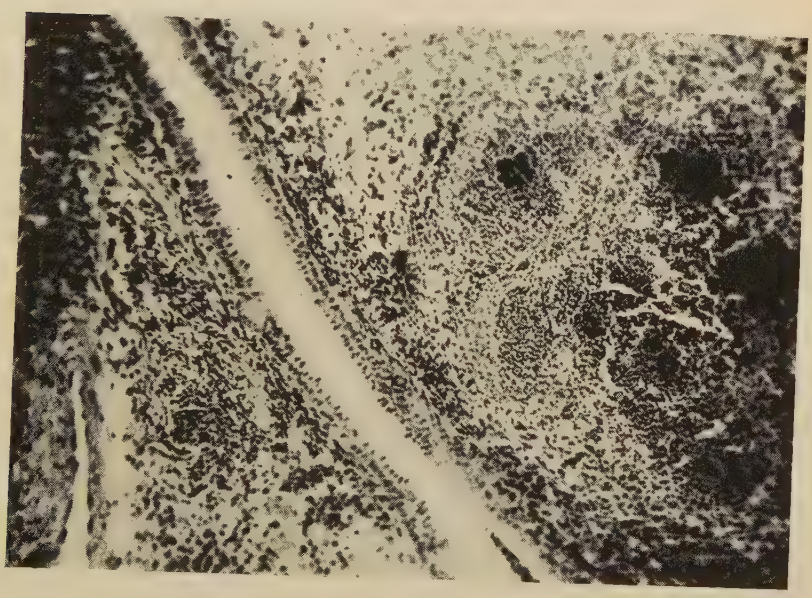

FIG. II. Actinobacillosis of lung. (× 125 approx.).

A—Granule.
B—Normal bronchiole.
C—Infiltration of round cells.
D—Artery.
E—Pus cells in bronchiole.

and sheep; serologically they are closely related but individual strains of both organisms may be subject to considerable antigenic variation: the former designation should therefore be discarded.

2. Actinobacillosis is widespread among sheep in Scotland, but the incidence in individual flocks is commonly low. The characteristics of the disease are described.

ACKNOWLEDGMENTS

The writer is indebted to Messrs. R. S. Masterton, J. Milne and J. B. Russell for material, to Dr. H. H. Holman for his advice on the histopathology of the disease, to Mr. J. B. Dow for the photographs, and to the Director of Moredun Institute, Dr. J. Russell Greig, for his continued interest in this work.

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[*Received for publication July 8th, 1944*]

AN OUTBREAK OF ULCERATIVE LYMPHANGITIS IN CATTLE CAUSED BY *CORYNEBACTERIUM OVIS*

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INTRODUCTION

The manager of a ranch in the Rift Valley noticed that a steer in a mob of 200 had a swelling on one side at the base of the neck which increased to the size of a duck's egg and burst within a week. The steer was slaughtered. Then another steer developed a similar swelling and was also destroyed. During the next month 21 steers became infected and were segregated. After the swellings had burst, a cautery was applied " to help dry up the weeping sores," but invariably other lesions developed near and associated with the original sites. It was at this stage, in July, 1943, that the disease was investigated by the author.

The steers were three to four years old, in good condition and grazing on rough stony country covered with thorny scrub. Those affected had one or more lesions usually only on one side, rarely on both sides ; when bilateral these were not symmetrical. In the early stages the lesions were raised swellings fixed to the skin or subcutaneous tissues. On careful palpation, a thickened cord was found connected with each swelling. All swellings were situated between the hindquarters and the base of the neck. When the areas drained by the precural or prescapular glands were affected the gland concerned was visibly enlarged, fibrous, and firmly attached to the surrounding structures. After the primary swellings had burst, an ugly ulcer remained with raised encrusted edges, from which a gelatinous translucent exudate oozed continuously. In some cases a chain of ulcers was strung on a thickened conspicuous lymphatic cord.

PORT-MORTEM EXAMINATION OF NATURAL CASES

On autopsy of the 21 affected steers that were slaughtered, it was possible to trace the course of the disease from the early closed swelling, the size of a pigeon's egg, to the later stages with ulceration and involvement of the lymphatic glands. From the primary focus in the form of a small abscess enclosed in a mass of fibrous tissue just under the skin, the organisms spread via the lymphatics which were grossly enlarged, turgid and cordlike, but did not contain visible pus, and eventually reached the gland draining the area. The first change in the gland was a general oedema and enlargement, then distinct necrotic foci developed, and on incising the gland one might see a white speck surrounded by a tough fibrous zone. At a later stage one or more collections of cream-coloured semi-liquid lardaceous pus might be seen containing firmer caseous lumps. Abscesses containing up to two ounces pus were found, but no

gland was actually ulcerated. A carcase with advanced disease was fully dissected ; the precural glands on one side contained large abscesses and infection had spread into the superficial and deep inguinal glands. In this animal as in other affected animals all the visceral, thoracic and head glands were free of infection. Immediately they were detected and before any lesion burst all diseased animals were sent to the abattoirs or slaughtered on the ranch. This prompt action appeared to have been all that was necessary to eliminate the disease from the herd, for after two months no further cases occurred.

MICROSCOPIC EXAMINATION OF BOVINE MATERIAL

Pus smears showed few scattered gram-positive but no acid-alcohol-fast bacilli. Smears were afterwards carefully searched for gram-positive bacilli, since cultures from the first case had grown a diphtheroid. Gram-positive bacilli were found in smears from every carcase, but they were often very rare. In gland smears they varied from 1 to 3 μ by 0·5 μ length, and were rounded at the ends ; they were usually single and only rarely in short chains, but they often occurred as compact groups of rods. Affected gland sections showed foci of necrotic pus cells surrounded by much fibrous tissue, and in areas away from the actual site of infection there was a marked cellular activity with increase in the size of the lymph germ centres resulting in a compensatory reduction in the sinusoidal areas.

CULTURAL CHARACTERS

The organism was isolated by sowing pus on ox-blood agar and incubating for 24 hours at 37° C. Sixteen out of the 19 affected animals examined yielded *C. ovis* by this method.

Ox Blood Agar.—After 24 hours' incubation white, opaque colonies were just visible which increased in size and after several days incubation grew up to 5 mm. in diameter. At first the colonies were smooth, shiny, and porcelain-like, but after the second day the centre became raised, the edge slightly scalloped and faint radial depressions appeared. Around each colony there was a clear zone of β. haemolysis. Carne (1939) gives a photograph of a very typical colony. In primary cultures small clear minute convex colonies could be seen between the colonies just described ; on sub-cultivation they gave rise to the ordinary large type. All growths were very friable and tended to detach bodily from the agar on touching with a platinum needle. A smooth suspension could not be obtained in saline or water, hence smears from cultures on solid media showed the bacilli in clumps.

Loefflers Inspissated Serum.—Satisfactory growths were obtained, the colonies appearing moister smooth, and light cream in colour. The growth in the fluid at the bottom of moist slopes was brilliant yellow.

Serumised Peptic Digest Broth.—Growth was abundant, precipitating in yellow granular clumps while the supernatant remained clear. There was also a very friable surface growth breaking up and depositing at the slightest movement.

Serumised (5 *per cent.*) *Gelatine.*—The organism grew well in this medium at 37° C. After 16 days' incubation the gelatine set on cooling to 19° C. indicating that little or no liquefaction had taken place. Stab cultures left at room temperature showed a fine growth along the needle track after two days, which became more definite, and after a week a minute cup-like depression was formed. In this crater-like depression the growth extended to the rim and was dry and wrinkled.

Agar, Broth, Gelatine, and Peptone Broth without Serum.—In these media, without serum enrichment, growth was poor : on the solid media it was dry and the colonies were pin-point in size.

Biochemical Reactions.—The fermentation reactions studied in serumised (10 per cent.) peptone water, with Durham tubes and Andrade's indicator, were : acid but no gas in glucose, sucrose (slow), maltose, galactose (weak), laevulose (slow), and dextrine ; no change in lactose, manite and dulcite. Tested on sugared serum agar slopes, with litmus as an indicator, the results were identical with those given above for liquid sugars.

MORPHOLOGY AND STAINING REACTIONS OF THE ORGANISMS FROM CULTURES

The bacilli were remarkably even in size and shape whether grown on solid or in liquid media. Stained smears often revealed the organisms lying in parallel groups, giving an appearance not unlike a loose bundle of sticks. Meta-chromatic granules and bars were seen in Neisser's stained preparations and more frequently so in growths on inspissated serum. Barred bacilli were a very small minority of the total organisms. In old cultures, there were some club-headed organisms present as also other less regular forms.

ANIMAL INOCULATIONS

Cattle.—Four young steers were inoculated with 24 hours' growth in serum peptone broth with results shown in Table I.

Bovines 4693 and 5370 infected by inunction had a slight thickening of the skin on the scarified areas, and after four days a scab formed. They showed no rise in temperature and remained healthy throughout the period of observation. The two beasts 4646 and 5367 inoculated subcutaneously, reacted sharply. By the third day they were listless and had temperatures of over 106° F. and the sites of inoculation were swollen (10 by 10 in.) oedematous and painful. No. 5367 having lost condition, was killed on the 5th day, and *post-mortem* examination revealed that the swellings were due to the infiltration of the tissues with bright yellow lymph. The muscles and connective tissue in this area were saturated and the lymph exuded

freely from the cut surfaces. No organisms could be detected in smears made from the fluid, and cultures were sterile. The other steer (No. 4646), having regained condition after the severe reaction, was slaughtered 73 days after inoculation and autopsy showed that it had completely recovered.

Sheep.—Five sheep (Nos. 7033, 7034, 7038, 7045 and 7089) received 5 cc. of 24 hours' broth culture subcutaneously in the thigh, and all died two to four days after infection, *post-mortem* showing intense icterus and lysis of the blood cells. In all, the temperature rose on the second day (104·2 to 107° F.) and the animals were listless, and passed red urine. When blood was collected at this stage and allowed to coagulate, the clot formed was very small and the serum dark crimson. Most of the red cells were haemolysed. After death, the whole carcase was markedly yellow. The abdominal and pleural cavities contained about 500 cc. of blood-stained fluid. The liver was greyish-yellow, peppered with lighter yellow areas.

TABLE I

No.	*Mode of Inoculation.*	*Reaction.*	*Post-mortem Examination.*
4693	Inunction of scarified area	No infection	Not done, animal healthy
5370	Ditto	Ditto	In 73 days after infection, no lesions
4646	Subcutaneous inoculation into shoulder and neck 1 c.c.	Large local swelling, temperature raised	In 73 days, no lesions
5367	Ditto	Ditto	In 5 days, extensive oedema with yellow transudate

The spleen was engorged and dark. The kidneys were flabby with a bright pink cortex. Around the inoculation site there was an intense haemorrhagic oedema within a radius of two inches. The local lymphatic glands were haemorrhagic and oedematous. *C. ovis* was isolated from the point of inoculation but not from any other organ or tissue. Every carcase on removal of the skin turned to a brilliant salmon pink after an hour's exposure.

Similar fatal results were obtained when washed bacilli were inoculated. On the other hand, the filtrate obtained from a culture passed through a Seitz (EK) pad produced no ill-effects when inoculated into sheep or guinea-pigs.

Norgaard and Mohler (1899) injected a sheep with 1 cc. of a two days' culture of the organism of caseous lymphadenitis and the sheep died on the fifth day. Carré and Bigoteau (1908) in their report on a sheep which died three days after the subcutaneous inoculation of 1 cc. of " toxin " derived from the filtering of a culture of the Preisz-Nocard bacillus through Chardin paper or a " *bougie Berkefeld très poreuse*," remarked on the general yellowness of the corpse, and stated, " *le sang des capillaires est rose vif.*"

Guinea-pigs.—Using Hewlett and Bulloch's (1930) technique for assessing the virulence of *C. diphtheriae*, three strains of *C. ovis*

isolated locally from bovine, caprine, and equine (ulcerative lymphangitis) material, were compared by injecting them intracutaneously (inoculum 0·2 cc.) into three groups, each of two guinea-pigs with the following results :—

Bovine Strain. Guinea-pig 1. Died three days p.i.* At s.i.† blood clot 1 × 1 in. Little oedema. Blood-stained fluid (20 c.c.) in abdomen. Adrenals enlarged ; the left had a few petechiae on surface, medulla very dark, cortex white. Kidneys congested and dark. Lungs, oedema. Stomach, mucosa diffuse red. Testes normal. Whole carcase yellow. *C. ovis* recovered from s.i.

Guinea-pig 2. Died six days p.i. Pus at s.i. Adrenals, enlarged and pink. Kidneys, congested. Epicardial haemorrhages. Stomach and intestinal walls, thickened with oedema. Lungs, petechiae and oedema. Carcase pink. *C. ovis* recovered from pus.

Horse Strain. Guinea-pig 3. Died three days p.i. *Postmortem* findings similar to guinea-pig 1. Guinea-pig 4. Survived 60 days, apparently complete recovery.

Goat Strain. Guinea-pig 5. Died 24 days p.i. Cachexia with ulcer 3 × 3 c.m. at s.i. Abscess in local gland and spleen. Cultures, *C. ovis* from abscesses. No lesions in testes.

Guinea-pig 6. *Postmortem* findings as in guinea-pig 5.

Within an hour of inoculating these guinea-pigs, an area one inch in diameter around the s.i. was raised and doughy. After 20 hours the centre had a dry necrotic scab 0·5 c.m. across. In those animals that survived ten days or longer, a discharging ulcer developed with visible thickened corded lymphatics running into it. In the two guinea-pigs which had received the goat strain, secondary abscesses developed along the lymphatics giving a general picture closely resembling that of equine ulcerative lymphangitis.

Mice.—Four male white mice were each given 0·5 cc. of 24 hours' broth culture intramuscularly into the thigh.

Mouse 1. Died 17 days p.i. Cachexia ; inoculated limb, dry and withered. Testicles enlarged and dotted with minute abscesses. Cultures from testes yielded *C. ovis*.

Mouse 2. Killed *in extremis*, 17 days p.i. Testes normal. Abscesses in precrural gland and spleen yielding *C. ovis* on culture. Withered leg.

Of the remaining mice, one died 21st day p.i. with *postmortem* findings as M.1. The other was killed *in extremis* 21st day p.i. Cachexia, withered limb. Testes normal. Culture from s.i. yielded *C. ovis*.

Dog.—A dog was inoculated in the saddle area with 2 cc. of a 24 hours' serum-broth culture. During the next four days the temperature rose to 103° F., a local oedematous plaque (3 by 4 in.) developed which dispersed by the 21st day leaving a small discharging ulcer. This healed completely after four weeks. The dog did not lose condition at any time and completely recovered.

DISCUSSION

In its morphological, staining, cultural and biochemical characters and its pathogenicity for animals, the organism is identical with

*p.i.—after inoculation. †s.i.—site of inoculation.

C. ovis as described by Carne (1933) but no outbreaks of natural infection resembling the one here described have been traced in the literature. Carne (1939) states, " There are reports in the literature of the occurrence of natural infection by *C. ovis* in the ox, goat, camel, wild deer, pig and fowl. Most of these papers, however, are unconvincing." Of 134 strains studied by Carne, only one was from a bovine. Bull (1933) reported a cow with an ulcerating injury from which *C. ovis* was isolated by cultural means. It is interesting to note that he recorded the discharge as " a clear transudate," and also that she recovered. The most likely method of natural infection of sheep with *C. ovis* is by ingress of the organism through surface wounds. McGrath (1929) reported the recovery of *C. ovis* from recent shear cuts. Seddon and his co-authors (1929) found a correlation between the common sites of shearing injuries and the location of caseous lymphadenitis whilst Woodruff and Oxer (1929) suspected the umbilicus of new-born lambs as another possible site for the entry of *C. ovis*.

The distribution and superficial nature of the primary lesions of the bovine outbreak here recorded together with the work on natural infection in sheep by the authorities just quoted, suggest strongly that these cattle became infected with *C. ovis* through scratches and other minor skin injuries. This explanation is probably correct since the cattle were grazing in thorny bush country. As *C. ovis* infection of sheep and goats is not rare in Kenya this organism must be widely distributed in this territory. Bull and Dickinson (1933) isolated *C. ovis* from soil collected from areas in which caseous lymphadenitis was known to be prevalent.

Carpano (1934) described an " ulcerative dermatitis of cattle " from which he isolated *C. diphtheriae*. The photographs illustrating his paper show an affected cow with lesions very similar to those found in the cattle described in this communication. Daubney (1927) recorded the finding of tropical actinomycosis of cattle in Kenya, the causal organism being an acid-fast actinomyces. Further, the clinical picture of this condition is distinct. Bovine tropical actinomycosis is a chronic systemic condition involving most, if not all, the lymph glands, whilst infection of bovines by *C. ovis* is a less severe and more localised disease.

Carré and Bigoteau (1908) reported a disease of sheep known as " Mal Rouge " due to *C. ovis*. Robinson (1928), whilst investigating mortality in sheep following bluetongue inoculations, recovered *C. ovis* from abscesses and by the inoculation of this organism into sheep reproduced the intense haemolytic condition which he named " Bacterial icterus." His attempts to reproduce " Bacterial icterus " by injecting healthy sheep with the blood of an affected animal were unsuccessful. In the writer's experiments, media sown with haemolysed blood of sheep dying after inoculation with *C. ovis* were sterile. It appears that the red cells of a living sheep are destroyed by a haemolytic agent produced by the organisms at the site of inoculation, and it is logical to assume that this factor must

be soluble since otherwise it would be difficult to account for the rapid destruction of the erythrocytes. Nevertheless, it is remarkable that an organism that produces such violent and frequently fatal lysis of sheep cells *in vivo*, should seldom produce an exo-haemolysin when grown *in vitro*. Many attempts were made to detect haemolysins against sheep's cells with these strains of *C. ovis* from cattle. The procedure followed was that evolved by Lovell (1937) for *C. pyogenes* toxins, and serum broth was used in place of cooked meat medium, but no haemolysins against sheep's red cells were found.

It is interesting to record that this disease of cattle due to *C. ovis*, is known to the Masai tribesmen, who firmly believe that rhinoceroses are responsible for introducing it into cattle herds. Chronic ulcerating sores are commonly found on rhinoceroses, but no proof is as yet available that these sores are caused by, or contain *C. ovis*.

SUMMARY

An outbreak of ulcerative lymphangitis associated with infection with *Corynebacterium ovis* in a herd of cattle in the Rift Valley, Kenya, is described. The characteristics of the organism are examined and discussed.

ACKNOWLEDGMENT

I gratefully acknowledge the help rendered by Mr. R. Daubney, the Director of the E.A.C.V.R.I. in the preparation of this paper and for permission to publish it.

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[*Received for publication June 28th, 1944*]

PENTOTHAL SODIUM AS A NARCOTIC IN CATTLE

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INTRODUCTION

In the course of experimental work at this Research Station it was necessary to perform some minor operative procedures in cattle. These required more restraint of the animal than could be obtained without the aid of a narcotic or an anaesthetic. It was important that induction of narcosis should be rapid, that recovery should be prompt and unattended by excitement or struggling. The intravenous administration of pentothal sodium [sodium ethyl (1-methyl-butyl) thiobarbiturate, Abbott] is used extensively in human medicine to meet these requirements, and although its successful employment in dogs and cats has been reported [Sweebe and Khuen (1936): Sweebe (1936); Wright (1937 and 1942); Sheppard, M. and D. H. (1937)] no record has been found of its use in adult cattle. Wright (1942) reports satisfactory results in young calves, 10 to 14 days old, slow intravenous injection of a 6·5 per cent. solution producing light anaesthesia which persisted for 10 to 12 minutes after which recovery was rapid. Consciousness was fully regained in 30 minutes, but some inco-ordination of limb movement remained for about two hours.

In human medicine the drug is administered very slowly, the dose given gauged according to the response of the patient, anaesthesia being maintained by intermittent injections of small amounts. Administration to large animals is more convenient if the required dose can be given quickly at one time for, unless the animal is restrained in a lying position before the injection is commenced, the act of falling to the ground is almost certain to displace the needle from the vein.

EXPERIMENTAL DETAILS

Experimental animals.—All the animals used were Devon steers in store condition, from 1½ to 2 years old. Their estimated weight was 600 to 800 lb.

Preparation of the animals.—The cattle used in these experiments were housed in loose boxes and were fed twice daily. They were not specially prepared in any way, and the drug was administered from two to seven hours after feeding.

Preparation of the solution for injection.—The drug is supplied as a crystalline powder in ampoules of 0·5 and 1 gm.; 20 c.c. of chemically pure water is provided for solution of each gramme. The solution is freshly prepared before use by adding the water to the ampoule containing the powder. On one occasion, when a number of cattle were going to be injected, the contents of the required number of ampoules were dissolved and the solution pooled in a

flask. Less than one-and-a-half hours elapsed before all the solution was used, but the results obtained were less satisfactory than usual. In view of the unstable nature of the solution, it is recommended that the dose for each animal be made up immediately before injection.

Strength of solution.—In man it is advised that the strength of the solution should not exceed 5 per cent., as with stronger solutions there is a risk of thrombophlebitis. This risk is less in cattle especially if the jugular vein is selected for injection as the greater volume of blood leads to quicker dilution of the drug. It has been found that a 10 per cent. solution can be used with safety. The various strengths of solution used are shown in Table 1.

Administration and effect produced.—In every case the drug was administered by rapid injection into the jugular vein while the animal was in the standing position. From 1 to 4 gm. have been given and the effects produced are summarised in Table I. When

TABLE I

EFFECT PRODUCED BY THE RAPID INTRAVENOUS INJECTION OF PENTOTHAL SODIUM

Dose and Strength of Solution Administered.	*Number of Observations.*	*Effect of the Drug.*			
		Muscular Inco-ordination but no Narcosis.	*Light Narcosis.*	*Deep Narcosis.*	*Light Anaesthesia.*
1 gm., 5 per cent.	1	1	—	—	—
2 gm., 5–10 per cent.	13	3	3	6	1
3 gm., 7·5–10 per cent.	32	—	2	29	1
4 gm., 10 per cent.	1	—	—	1	—

narcosis was induced the result was immediate, the animal falling to the ground within a few seconds of completion of the injection, the full effect being apparent within one to two minutes. The state of deep narcosis was characterised by shallow respirations, a slight slowing of the reflex response to stimulation of the cornea and anus and no resentment to forced movements of the limbs or head. This state persisted for 5 to 10 minutes after which recovery was quick, with 2 gm. the animals had regained consciousness and assumed the normal bovine recumbent position in from 10 to 20 minutes, with 3 gm. this took from 25 to 35 minutes. The 2 gm. dose prevented the animals rising for from one to two hours, and 3 gm. for from two to three hours. At this stage some slight inco-ordination of limb movement persisted, but it is noteworthy that there was a complete absence of struggling during recovery, restraint was never necessary to prevent the animal injuring itself during this period. If an animal attempted to rise and failed, it was content to rest quietly until recovery was complete.

It will be seen that the effect produced in adult cattle is very similar to that described in young calves by Wright.

DISCUSSION

Pentothal sodium has proved to be a most useful drug for the purpose for which it was selected by the author, and there is no doubt that its action may have a wider application in the field of general veterinary practice either as a narcotic alone or as a preliminary to inhalation anaesthesia. The great advantage that it has over other non-volatile anaesthetics is the rapidity and peacefulness of recovery. For the induction of deep narcosis in cattle of the weight used it is better to give 3 gm. of the drug as this was successful in 30 out of 32 instances, whereas 2 gm. failed to produce deep narcosis in 6 animals out of 13.

Although two of the cattle in these experiments passed beyond the stage of narcosis to that of general anaesthesia (see Table 1) no attempt has been made to determine the amount of the drug necessary to induce and maintain general anaesthesia as narcosis was sufficient for the purpose. On analogy with the action of the drug in other subjects it is probable that intermittent injections of much smaller amounts than are necessary to induce anaesthesia would be sufficient to maintain it.

The principal drawback to the general use of pentothal sodium in large animals is the cost of the drug: at the present level its application will perforce be confined to selected cases.

SUMMARY

1. A description is given of the successful intravenous use of the barbiturate pentothal sodium as a narcotic in cattle.

2. A dose of 3 gm. is required for the induction of deep narcosis in Devon steers of 800 lb.

3. Induction of narcosis is immediate and recovery is rapid, being unattended by any struggling so that restraint is not necessary during this period.

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[*Received for publication July 17th, 1944*]

THE USE OF THIONINE BLUE FOR THE IDENTIFICATION OF *BRUCELLA ABORTUS* STRAIN 19

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INTRODUCTION

The widespread use of the Ministry of Agriculture's *Brucella abortus* Vaccine No. 1* makes it desirable to possess an easy method of identifying this strain. The method described in this article is put forward as being practical, and as being quicker and less costly than the inoculation of guinea-pigs.

Huddleson (1934) suggested the use of thionine blue as a means of distinguishing *Brucella abortus* from both *melitensis* and *suis*, because of the greater sensitivity of the former to the bacteriostatic action of the dye. Other workers have reported that individual strains of *abortus* may be as resistant to thionine blue as the other two species (Van der Schaaf and Rosa, 1940 : Mingle and Manthei, 1941), but no strain has so far been reported as being even more sensitive to thionine blue than normal strains of *abortus*.

The writer found strain 19 to be highly sensitive to thionine blue and other dyes, and suggests the use of thionine blue as a means of identifying this strain of the organism.

METHOD

A series of Petri dishes is prepared containing solid medium including a range of concentrations of the dye under examination. Suspensions of the strains of *Brucella abortus* to be tested, containing approximately the same number of organisms per unit volume, are prepared and one loopful of each is rubbed on to approximately one square centimetre of surface of each of the plates in the series. A plate containing medium with no dye is included as control. The suspensions should preferably be made in sterile tap-water as this is known to preserve the viability of the organisms over considerable periods, and should be made from fresh growths of the same age, as aged cultures appear to vary greatly in their sensitivity to the basteriostatic action of dyes.

After sufficient incubation, in an atmosphere containing approximately 10 per cent. of carbon dioxide, to produce good growth of all strains on the control medium, the series is examined and the highest concentration of dye permitting macroscopic growth of each strain is determined. This concentration is subsequently denoted as the titre for a strain in respect of a particular dye.

* Prepared from *Br. abortus* (Strain 19), Bureau of Animal Industry, U.S.A.

RESULTS

Selection of Dye.—A series of twelve bacteriostatic dyes was examined and five were found to inhibit the growth of strain 19 at concentrations much lower than those required to inhibit three other strains of *Brucella abortus* (see Table I).

In the case of methylene blue and thionine blue the " differential bacteriostatic ratio " (calculated simply by dividing the highest concentration of dye permitting the growth of the other strains by that permitting the growth of strain 19) was as high as 16 : 1. Hence thionine blue, already widely in use for this type of work, was selected for more detailed and extensive examination.

TABLE I

SENSITIVITY OF FOUR STRAINS OF *Brucella abortus* TO FIVE BACTERIOSTATIC DYES

Dye.	*Titre**		*" Differential Bacteriostatic Ratio."*
	Strain 19.	3 *Other Strains*†	
Basic fuchsin ...	140 mg./litre	280 mg./litre‡	At least 2 : 1
Safranin	40 „	320 „ ‡	„ 8 : 1
Acriflavine ...	0·25 „	2 „	8 : 1
Methylene blue ...	0·25 „	4 „	16 : 1
Thionine blue ...	0·25 „	4 „	16 : 1

* Highest concentration in mg. of dye per litre of medium allowing of growth of inoculum of about 50 organisms.

† Strains 544, Galston and 45.20 (see Table IV).

‡ Highest concentration of dye used.

Selection of Medium.—Obviously a medium must be used which supports the growth of all strains of *Brucella abortus*. Owing to the difficulty of obtaining liver medium, substitutes must be found. Blood agar, while excellent for isolation work, does not support very strong growth, especially in the presence of dye. Bactotryptose agar produces rapid and heavy growth, and with the addition of blood is an excellent substitute for liver medium. In this instance, however, it appears advisable to omit the blood, as it interferes in some way with the strong bacteriostatic action of thionine blue on strain 19, as is apparent in Table II.

TABLE II

EFFECT OF THE ADDITION OF BLOOD TO BASAL BACTOTRYPTOSE AGAR MEDIUM ON THE BACTERIOSTASIS OF STRAIN 19 BY THIONINE BLUE

Percentage of Blood Added.	*Titre.*		*" Differential Bacteriostatic Ratio."*
	Strain 544.	*Strain* 19.	
0	5·6 mg./litre	0·35 mg./litre	16 : 1
1	5·6 „	0·5 „	11 : 1
5	5·6 „	0·7 „	8 : 1

Selection of Inoculum.—The inoculum generally used in *Brucella* dye work is a comparatively heavy one, one loopful of a suspension

approximately equivalent in opacity to twice that of Brown's tube 10 being generally used. From such an inoculum of strain 19, however, growth may occur in concentrations very much higher than those that just permit the growth of light inocula.

With a culture of strain 19 obtained from dried material, the titre is very nearly the same for any size of inoculum up to 50,000,000 organisms per sq. cm. of surface of medium. When however, the strain is maintained in subculture for any length of time, the titre of a heavy inoculum gradually increases to a high figure. With a light inoculum of about 50 organisms, on the other hand, after cultivation for a period of as long as twelve months, the titre remains constant at the same low figure as obtained for the fresh strain.

Table III illustrates this phenomenon, which, for reasons that do not come within the scope of this article, is considered by the writer to be occasioned by a type of bacterial dissociation. On account of it, however, it is thought advisable to use comparatively light inocula, as these always illustrate clearly the greater sensitivity of strain 19 to dyes.

TABLE III

TITRES FROM VARYING INOCULA OF STRAINS 19 AND 544 AT VARYING AGE OF CULTURE

Strain.	*Age of Culture.*	*Number of Organisms Inoculated per Sq. cm. of Surface of Medium.*		
		50	50,000	50,000,000
19	Freshly recovered from dried material... ...	0·25 mg./litre	0·25 mg./litre	0·5 mg./litre
19	Subcultured over a period of three months ...	0·25 „	0·25 „	2 „
19	Subcultured over a period of six months ...	0·25 „	1 „	5 „
19	Subcultured over a period of twelve months ...	0·25 „	2–5 „	5–10 „
544	Freshly recovered from dried material... ...	2 „	5 „	10 „
544	Subcultured over a period of twelve months ...	2 „	5 „	10–20 „

Efficiency of Thionine Blue.—Sixteen strains of *Brucella abortus*, of varying character, under varying experimental conditions, and obtained from widely divergent sources, were submitted to examination for their sensitivity to the bacteriostatic action of thionine blue. As can be seen from Table IV, all except strain 19 were very nearly equally sensitive to the dye, while strain 19, under all conditions, was sensitive to at least one-eighth of the concentration required to inhibit all the other strains.

Tests on a selected few strains, including strain 19, have been carried out repeatedly over a period of twelve months, always with the same result. The " differential bacteriostatic ratio " for strain 19,

TABLE IV

THIONINE BLUE SENSITIVITY OF 16 STRAINS OF *Brucella abortus*

Strain	*Character*	*Source and Experimental Treatment*	*Average Titre**
Veronica ...	Virulent, smooth, CO_2-sensitive	Recent isolation from aborting heifer (Berkshire)	2·8 mg./litre
" ...	" " "	Re-isolated from guinea-pigs	2·8 "
Simmonds ...	" " "	Recent isolation from aborting heifer (Berkshire)	2·8 "
" ...	" " "	Re-isolated from guinea-pigs	2·8 "
Galston ...	" " "	Recent isolation from aborting heifer (Ayrshire)	2·8 "
" ...	" " "	Re-isolated from guinea-pigs	2·8 "
Motton ...	" " "	Recent isolation from aborting heifer (Penzance)	2·8 "
" ...	" " "	Re-isolated from guinea-pigs	2·8 "
Hereford II ...	" " "	Recent isolation from aborting heifer (Hereford)	2·8 "
" III ...	" " "	" " " (")	2·8 "
Cow II ...	" " "	Isolated through guinea-pigs from udder of cow 14 days after injection of strain 45.20	2·8 "
544 (McEwen)..	" " "	Dried culture of stock strain originally obtained from aborting cow	2·8 "
" ...	" " "	Re-isolated from normal guinea-pigs up to 60 days after injection	2·8 "
" ...	" " "	After repeated guinea-pig passage (up to five passages)	2·8 "
" ...	" " "	Re-isolated from aborting guinea-pigs	2·8 "
" ...	" " "	" " " heifers	2·8 "
" ...	" " aerobic	After repeated subculture on artificial medium	2·8 "
Human ...	" rough	Human case of " *abortus* fever "	2·8 "
1785	? "	(Unknown)	2·8 "
Boots	? "	(")	2·8 "
D3R	Avirulent, rough, CO_2-sensitive	Recovered from aborting heifer vaccinated with strain 45.20 and injected with strain 544	2·8 "
Cow V... ...	" " "	Isolated from cow aborting 45 days after injection of strain 45.20	2·0 "
45.20 (McEwen)	" " aerobic	Dried culture of stock strain (see McEwen, 1940†)	2·0 "
"	" " "	Recovered from fresh Vaccine No. 2 (Ministry of Agriculture)	2·0 "
"	" " "	Recovered from expired Vaccine No. 2	2·0 "
"	" " "	Re-isolated from normal guinea-pigs up to 60 days after injection	2·8 "
"	" " "	After repeated subculture on artificial medium	2·0 "
D4	" smooth, "	Recovered from heifer vaccinated with strain 45.20 and injected with strain 544 during pregnancy	2·8 "
19	" " "	Dried culture of stock strain (from Ministry of Agriculture) (three samples)	0·25 "
19	" " "	Recovered from fresh Vaccine No. 1 (Ministry of Agriculture)	0·25 "
19	" " "	Recovered from expired Vaccine No. 1	0·25 "
19	" " "	Re-isolated from guinea-pigs up to 16 days after injection and after serial guinea-pig passage	0·25 "
19	" " "	After repeated subculture on artificial medium	0·25 "

* The titre varies between different assays, but in any one assay the " differential bacteriostatic ratio " between strain 19 and any other strain has never fallen below 8 : 1.

†McEWEN, A. D. (1940). *Vet. Rec.*, **52**, 815.

while varying in individual assays to as high as 45 : 1, has never fallen below 8 : 1.

This ratio is sufficiently high to permit of the preparation of a medium containing just sufficient dye to inhibit the growth of strain 19 without affecting any other strain of *abortus*, and the practical method proposed below has been used with success, not only in detecting simply the pure strain, but also in separating it from a mixture recovered, from local lymph glands, up to four days after inoculation of guinea-pigs with equal inocula of strain 19 and of a fully virulent strain.

PRACTICAL USE OF THE DYE TEST

On account of the variability of dyes and of media used in different laboratories, a test series of dye concentrations must be made and inoculated with both strain 19 and some other strain, in order to determine that concentration at which strain 19 will be inhibited and the other capable of growth ; it is advisable to use light inocula, such as would give rise to discrete growth.

In the present case, 1 mg. of thionine blue (B.D.H. 168626/390505) per litre of bactotryptose agar was satisfactory.

Colonies from isolation plates are picked and rubbed up in sterile tap-water to form a dilute suspension. This is inoculated on medium containing dye and on medium free from dye, the inoculum being spread over a small area of the surface of the medium. (In this way it is possible to test as many as 50 strains on one 4-inch plate).

Growth on the control medium, but not on the medium containing dye, indicates a strain highly sensitive to the action of thionine blue. The only strain of *Brucella abortus* of this type, at present known, is strain 19.

SUMMARY

(1) Strain 19 (the Ministry of Agriculture's *Brucella abortus* Vaccine No. 1) is much more sensitive to the bacteriostatic action of certain dyes than fifteen other strains of *abortus* examined.

(2) A test for the differentiation of strain 19 from other strains is suggested, using a single predetermined concentration of thionine blue, in solid medium, which will inhibit strain 19 but not other strains.

(3) As opposed to the Huddleson technique, a very light inoculum is employed as complications are introduced when heavy inocula are used, owing to what is considered to be a type of bacterial dissociation in strain 19.

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[*Received for publication August 10th, 1944.*]

Printed in Great Britain by H. R. GRUBB, LTD., Croydon